Introduction
Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar. Hyperglycaemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels.

"Diabetes mellitus" of any type develops when the body cannot produce as much insulin as it needs, resulting in high blood glucose levels (Gale 2005). Diabetes used to be considered one disease. Then it was divided into two main types called "juvenile" and "adult onset." These were later renamed "insulin dependent diabetes mellitus (IDDM)" and "non-insulin dependent diabetes mellitus (NIDDM)." Now they are called "type 1" and "type 2." Complications from any type of diabetes may include neuropathy (nerve damage), retinopathy (eye damage), nephropathy (kidney damage), and premature death. These may occur even with good glucose control (Narendran et al., 2005). At present, Diabetes Mellitus has become the third human killer after Cardiovascular, cerebrovascular diseases and Cancer.

Global Prevalence of Diabetes Mellitus

The estimated diabetes prevalence worldwide for 2011 was 366 million and it is expected to affect 552 million people by 2030. The International Diabetes Federation (IDF) estimated that in 2011 the five countries with the largest numbers of people with diabetes were China, India, the United States of America, Russia and Brazil. The IDF also reported that in 2011 the five countries with the highest diabetes prevalence in the adult population were Kiribati, Marshall Islands, Kuwait, Nauru and Lebanon. Low and middle income countries face the greatest burden of diabetes (Welsh Health Survey 2010 national statistics). The number of people with type 2 diabetes is increasing in every country. 80% of people with diabetes live in low- and middle-income countries. The greatest number of people with diabetes is between 40 to 59 years of age. 183 million people (50%) with diabetes are undiagnosed. Diabetes caused 4.6 million deaths in 2011. Diabetes caused at least USD 465 billion dollars in healthcare expenditures in 2011; 11% of total healthcare expenditures in adults (20-79 years). 78,000 children develop type 1 diabetes every year (WHO’s World Health Statistics
2012) report, which includes data from 194 countries, states that one in three adults worldwide has raised blood pressure and one in 10 suffers from diabetes. In high-income countries, widespread diagnosis and treatment with low-cost medication have reduced mean blood pressure across populations, leading in turn to a reduction in deaths from heart disease. In Africa, however, more than 40 per cent of adults in many countries are estimated to have high blood pressure most of them remain undiagnosed, even though many of these cases could be treated with low-cost medications, which would significantly reduce the risk of death. In the case of diabetes, the global average prevalence is around 10 per cent, with up to one third of populations in some Pacific Island countries having this condition. Left untreated, diabetes can lead to cardiovascular disease, blindness and kidney failure.

An increase in obesity is also highlighted in the report as being a major health risk. “In every region of the world, obesity doubled between 1980 and 2008,” said the Director of the Department of Health Statistics and Information Systems at WHO, Ties Boerma. “Today, half a billion people – 12 per cent of the world’s population are considered obese.” The highest obesity levels are in the Americas, with 26 per cent of adults suffering from obesity, and the lowest in the South-East Asian region, where only three per cent of the population is obese. In all parts of the world, women are more likely to be obese than men, making them more vulnerable to diabetes, cardiovascular disease and some cancers (Wild et al., 2004).

According to WHO, non-communicable diseases currently cause almost two thirds of all deaths worldwide, in the numbers of deaths from heart and lung disease, diabetes and cancer. The countries with the largest number of people with diabetes are India, China, and the United States of America will be in 2025. The majority of people with diabetes in industrialized countries will be aged >65 years. On the other hand, the majority of people with diabetes in industrializing countries will be 45-65 years old. The countries estimated to have the highest number of people with diabetes in 2011 and 2030 are India, China, United States, Indonesia, Japan, Pakistan, Russian Federation, Brazil, Italy, Philippines and Bangladesh (Wild et al., 2004)
Table 1: Top countries for estimated number of people with Diabetes: 2011-2030

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<tr>
<th>S. No</th>
<th>COUNTRY/TERRITORY</th>
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Prevalence in India

India is facing a twin burden of under-nutrition and over-nutrition: it figures prominently both in the hunger map of the world as well as being the diabetes capital of the world (Mohan et al., 2007). A country experiencing rapid socioeconomic progress and urbanization, India carries the highest burden of diabetes with escalating prevalence in both urban and rural populations. India is facing an epidemic of diabetes, with high prevalence in urban areas. Over the past 30 years, the prevalence of diabetes has increased to 12-18% in urban India and 3-6% in rural India with significant regional variations. (Mohan et al., 2007) These rates in India are 50-80% higher than China (10%). The difference in prevalence of diabetes across India could be due to dissimilar levels of urbanization and lifestyle factors such as different diets and varying obesity levels (Ramachandran, 2002).

Significant determinants of diabetes are age, body-mass index (BMI), waist-hip ratio, low physical activity, and family history of diabetes. The driving forces behind the epidemic are urbanization (30%) and economic development with resultant increase in GDP, sedentary lifestyle, western diet, and fast food diet on a background of genetic susceptibility. The prevalence of both diabetes and prediabetes increases by age with 60% of Indians having diabetes or prediabetes by age 60 (Tuomilehto et al., 2001). While some genes confer increased susceptibility to diabetes among Indians; other genes that are protective in Europeans do not appear to protect Indians (Chandalia et al., 2005). Indians develop diabetes at a younger age and those younger than 45 years accounts for 36% of all diabetics in India (Ramachandran, 2002). Given that 80% of diabetics will die from cardiovascular disease (CVD), the medical and socioeconomic costs will be insurmountable unless urgent steps are taken to prevent or ameliorate the CVD complications which are higher among Indians than other populations (Shanthirani, 2007). CVD risk is primarily due to elevated lipids and blood pressure (much more than elevated blood sugar). Epidemiological studies show a significant and escalating burden of type 2 diabetes in India. This could be attributed to a high genetic risk and lower risk thresholds for acquired risk factors such as age, obesity, abdominal adiposity and a high percentage of body fat compared to Euripides (Ramachandran, 2002). Recent identification of genetic polymorphisms provides evidence of genetic predisposition to diabetes among Indians (Mohan et al., 2007). Asian Indians have higher central adiposity
which is an important determinant of several metabolic abnormalities, clinically referred to as metabolic syndrome. A large proportion of urban adults have the metabolic syndrome which predisposes them to both diabetes and cardiovascular diseases. Asian Indians accumulate a disproportionate amount of visceral fat so that the thin fat Indian baby grow up to be a thin fat Indian adult with thin legs and big belly (Mohan et al., 2007). Even modest amounts of weight gain during adulthood increases the risk of diabetes in Indians (Bhargava, 2007).

Both prediabetes and diabetes are the endpoint of long standing Insulin resistance, which appears to play a significant role for these conditions among Asian Indians. South Asians have decreased sensitivity to insulin when compared to other ethnic groups even in the absence of diabetes possibly due to comparatively increased levels of visceral fat resulting in a blunted response to insulin. Indians develop diabetes at least 10-15 years earlier compared to people of non-Indian origin (Bhargava, 2007). The prevalence of diabetes among the Indian Diasporas worldwide is greater than 20% and three to six times higher than the host population after standardizing for age (Bhargava, 2007). Asian Indians have the highest rates of diabetes in the US. The disease is more prevalent in southern regions as compared to northern and eastern parts of the country. Diabetes Epidemiology Study Group in India (DESI) investigators reported from several urban locations in India: age and gender-standardized prevalence of diabetes ranged from 9% in Mumbai to 12% Delhi, 12% Calcutta, 12% Bangalore, 14% Chennai and 17% in Hyderabad (Ramachandran, 2002).

WHO estimates that mortality from diabetes, heart disease and stroke costs about $ 210 billion in India in the year 2005. Much of the heart diseases and stroke in these estimates was linked to diabetes. WHO estimates that diabetes, heart disease and stroke together will cost about $ 333.6 billion over the next 10 years in India alone? The transition from a traditional to modern lifestyle, consumption of diets rich in fat and calories combined with a high level of mental stress has compounded the problem further. There are several studies from various parts of India which reveal a rising trend in the prevalence of type-II diabetes in the urban areas.

A National Urban Survey in 2000 observed that the prevalence of diabetes in urban India in adults was 12.1%. Recent data has illustrated the impact of socio-economic transition occurring in rural India and the transition has occurred in the last 15 years with the prevalence rising from 2.4% to 6.4%. Diabetes mellitus and impaired glucose tolerance
have reached epidemic proportions globally especially in developing countries like India. According to (King et al., 1993) in India the prevalence of diabetes in 1995 was 3.8% and it will be 6% by the year 2025. The number of diabetics in India in 1995 and 2025 are 19.4 million and 57.4 million respectively, placing India in the first position among the top 10 countries for estimated number of adults with diabetes in 1995 and 2025. The inevitable life style changes brought about by rapid industrialization and urbanization of the Indian society is thought to be the cause for this epidemic and the solutions for tackling this problem still remain elusive and expensive. But recent studies have shown that life style modifications can prevent or postpone the onset of diabetes in high risk population (Ramachandran et al., 2004). During the last two decades many population studies have been conducted in various parts of India looking at the prevalence of diabetes in south India was 8.2% and 2.4% in urban and rural population respectively. This was much higher than their own earlier estimates done two decades ago, which showed prevalence rates of 2.3% and 1.5% in urban and rural population respectively (Ramachandran et al., 2004).

The same researchers found even higher prevalence rates of 11.6% in their follow up study conducted 5 years later (Ramachandran et al., 2004). A recent large survey by (Saidikot et al., 2004) has shown that the age and gender standardized prevalence rates for Diabetes mellitus (DM) and Impaired Fasting Glucose in the total Indian population were 3.3% and 3.6%, respectively (urban DM prevalence 4.6% versus rural DM prevalence 1.9%).

**Diabetes on the Rise in Andhra Pradesh**

The American Diabetes Association, there are at least 31.7 million diabetic patients in India and the number is expected to grow to 79.4 million by 2030. In Andhra Pradesh alone about 30 lakhs of people suffer from diabetes and Hyderabad with its fast food joints and the Nawabi lifestyle is fast emerging on the world map of diabetes with many people joining the list of patients.

As the World Diabetes Day is observed on November 14, the World Health Organization cautions people that about 366 million people worldwide would be diabetic patients by 2030. A recent survey by the Diabetes Association of Andhra Pradesh showed that of the 12,000 people surveyed in rural areas, about two per cent or 240 people suffer from diabetes.
Types of diabetes

The three main types of diabetes are:

1. Type 1 diabetes.
2. Type 2 diabetes.

Type 1 Diabetes (Insulin-Dependent)

Type 1 diabetes, formerly called juvenile diabetes, is an autoimmune disease where the body's own immune system destroys the insulin-producing beta cells in the pancreas. People with type 1 usually have certain auto antibodies that may appear years before the disease develops, even in utero. These auto antibodies are used as markers of the disease, but do not necessarily cause the beta cell destruction. Some people, in fact, have these auto antibodies but never develop diabetes (Narendran et al., 2005).

A number of genes have been identified that are associated with the risk of developing type 1 diabetes. Some people, then, have a higher genetic risk than others, in other words, are more genetically susceptible. The genetic component of type 1 diabetes, however, is "neither sufficient nor necessary" (Vehik et al., 2008). That is, there is some environmental component to the disease: someone with high genetic risk might never develop it, while someone with low genetic risk might. More than 85% of the people who do develop type 1 diabetes do not have a parent or sibling with the disease (Larsson et al., 2004).

Type 1 has been divided into type IA and type IB, where type IA has an autoimmune cause, and type IB is "idiopathic" diabetes, that is, has no known cause. People diagnosed with type IB show signs of type 1 but have no evidence of autoimmunity (American Diabetes Association, 2011). A surprisingly high percentage (16%) of children and young adults newly diagnosed with type 1 diabetes in a Colorado diabetes center test negative for the antibodies associated with the disease. The younger the diagnosis, the higher the chance of auto antibodies being positive. Those who tested negative for antibodies had a higher body mass index, and may have a non-immune form of diabetes (perhaps different from either type IA or type 2) (Wang et al., 2010).
Type 2 Diabetes (Noninsulin-Dependent)

The most common form of diabetes is type 2 diabetes (once known as noninsulin-dependent diabetes mellitus or NIDDM). About 90 to 95 percent of people with diabetes have type 2 diabetes. This form of diabetes usually develops in adults over the age of 40 and is most common among adults over age 55. About 80 percent of people with type 2 diabetes are overweight.

In type 2 diabetes, the pancreas usually produces insulin (Florence and Yeager, 1999). But for some reason, the body cannot use the insulin effectively. The end result is the same as for type 1 diabetes—an unhealthy buildup of glucose in the blood and an inability of the body to make efficient use of its main source of fuel.

Diabetes is a disease that weakens the body’s ability to use food properly. The hormone insulin, produced in the pancreas, helps the body to change food into energy. In individuals with diabetes, either the pancreas does not make any insulin or the body does not use the insulin it has properly. The pancreas can be found on the left side of the body, in the abdomen below the stomach. It produces many digestive enzymes, insulin and glucagon, that break down food and hormones that regulate blood glucose (Gallagher-Allred C. 1999). When a person takes in a high load of sugar, the sugar stimulates the pancreas to release insulin. The targets for insulin are muscle, fat and liver cells. All these targeted cells have insulin receptor sites on the outside of the cell membrane. Type II diabetes is not a life threatening disease, but can cause numerous complications in one’s life because it is a lifelong disease that requires extraneous attention and care. For most people, when insulin has bound to the receptors, it causes a cascade of events to begin, which leads to the sugar being transported into the interior of the cell (Andallu and Varadacharyulu, 2003).

Diabetes is a very serious disease that attacks millions of people around the world. Diabetes is recognized as one of the leading causes of death and disability in the United States. It can strike at any age and can happen to anyone. Although we are not exactly sure about the causes of diabetes, it is known to be a metabolism disorder. It is believed that it has to do with the body's own immune system attacking and destroying insulin-producing cells in the pancreas. A metabolism disorder affects the digestion of food in the body. (McHenry, Robert1999) After eating, most food is broken down by glucose, which is the main fuel for the body. Cells use glucose for energy after it moves into the
bloodstream (Channing, 2008). Insulin, which is a hormone produced by the pancreas, allows the glucose to pass into our cells. The pancreas's job is to produce the right amount of insulin so the glucose can pass from the bloodstream into cells. (Channing, 2008) Without insulin the glucose that we need in order to live has a hard time entering the cells of the body that need it. If too much glucose builds up in the blood, then a diabetic may begin to have headaches or blurry vision.

**Gestational Diabetes**

Gestational diabetes develops or is discovered during pregnancy. This type usually disappears when the pregnancy is over, but women who have had gestational diabetes have a greater risk of developing type 2 diabetes later in their lives. Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2%–5% of all pregnancies and may improve or disappear after delivery. Gestational diabetes is fully treatable but requires careful medical supervision throughout the pregnancy. About 20%–50% of affected women develop type 2 diabetes later in their life (Chakravarti, 1997).

Even though it may be transient, untreated gestational diabetes can damage the health of the fetus or mother. Risks to the baby include macrosomia (high birth weight), congenital cardiac and central nervous system anomalies, and skeletal muscle malformations. Increased fetal insulin may inhibit fetal surfactant production and cause respiratory distress syndrome. Hyperbilirubinemia may result from red blood cell destruction. In severe cases, parental death may occur, most commonly as a result of poor placental perfusion due to vascular impairment. Labor induction may be indicated with decreased placental function. A cesarean section may be performed if there is marked fetal distress or an increased risk of injury associated with macrosomia, such as shoulder dystocia (Lanuzzo, 1982).

A 2008 study completed in the U.S. found that the number of American women entering pregnancy with preexisting diabetes is increasing (Dyer et al., 2008). In fact the rate of diabetes in expectant mothers has more than doubled in the past 6 years. This is particularly problematic as diabetes raises the risk of complications during pregnancy, as well as increasing the potential that the children of diabetic mothers will also become diabetic in the future.
Complications in pregnancy

Pregnancy poses additional risks for women with diabetes. The chances of having difficulties are greatly reduced through tight blood glucose control before and during pregnancy.

Babies of women with diabetes are

- Five times as likely to be stillborn.
- Three times as likely to die in their first months of life.
- Twice as likely to have a major congenital anomaly. This number could be higher as this figure is not adjusted for the higher rate of abortions in women where congenital abnormalities are found. The proportion of births to women with diabetes is rising due to an increased prevalence of Type 2 diabetes in younger people. (CEMACH 2007).

Other types of diabetes

Type 1, type 2, and gestational diabetes are the most common types of diabetes. Yet looking at the list provided by the American Diabetes Association, I count 48 other types of diabetes, plus "others." These include those that are drug or chemical induced, those caused by a genetic defect of the beta cells (such as MODY, Maturity Onset Diabetes of the Young), those caused by a genetic defect of insulin action, and more (American Diabetes Association, 2011). The classification of diabetes is more complicated than I had assumed, and distinguishing one from another can be difficult.

Genes and Environment

A large amount of research has focused on identifying gene variants that affect the risk of a person developing diabetes, as well as other diseases. These Genome-Wide Association (GWA) studies have identified a large number of genetic variants associated with various diseases. Yet most variants so far yield only small changes in risk levels. Only 6% of type 2 diabetes can be explained by heritable factors, for example (Manolio et al., 2010). GWAS studies are showing that "the magnitude of genetic effects is uniformly very small" (Dermitzakis and Clark, 2009).

Twin studies are also useful for looking at the role of genes. For type 1 diabetes, if one member of an identical twin pair has type 1, the majority of their twins do not
develop the disease, although the younger the diagnosis, the higher the risk. To assess the role of genetics, however, most twin studies compare the rates of disease between identical versus non-identical twins. Twin studies of type 1 show that disease risk is higher among identical twins than non-identical twins, showing a role for genetic background (Hyttinen et al., 2003). For type 2, a study from Denmark has found that the risk of type 2 diabetes does not differ between identical and non-identical twins (Petersen et al., 2011). Note that the environment, including the prenatal environment, is shared by both twins, if they are raised together, except that they may be exposed to different environmental factors during life, e.g., one might get a virus but the other does not.

An interesting study adapted the techniques used in GWA studies, and instead conducted a pilot Environment-Wide Association study to consider 266 separate environmental factors with diabetes, using a large U.S. dataset. It found that the factors most associated with diabetes include the pesticide heptachlor peroxide, PCBs, and a form of vitamin E. Protective factors included beta carotenes. The sizes of the effects that these factors have on type 2 diabetes are comparable to the highest risk gene loci found in GWA studies. This study is only the first EWA study ever done; there have been at least 16 GWAS studies on type 2 diabetes alone. Future studies might benefit from combining GWA and EWA data and methodologies, to consider the combined effects of genes and environment (Patel et al., 2010).

Symptoms of diabetes

Symptoms of Type I diabetes may include

- Increased thirst and urination
- Constant hunger
- Weight loss
- Blurred vision
- Extreme tiredness.

Without insulin, glucose can't get into body cells. Thus, even though blood sugar is high the body cells are starved. If not diagnosed and treated with insulin, a person can lapse into a life threatening coma. (Al-Khazraji, 1994)
Symptoms of Type 2 diabetes may include

- Feeling tired or ill
- Frequent urination (especially at night)
- Unusual thirst
- Weight loss
- Blurred vision
- Frequent infections
- Slow healing of sores.
- Having dry, itchy skin
- Losing feeling in the feet or having tingling in the feet

Insulin is usually present in the blood and some glucose gets into body cells. The symptoms of type 2 diabetes develop gradually and are not as noticeable as in type 1 diabetes (Alberti and Zimmet, 1998).

Complications of Diabetes Mellitus

Diabetic Ketoacidosis

Diabetic ketoacidosis (DKA) is an acute and dangerous complication that is always a medical emergency. Low insulin levels cause the liver to turn fatty acid to ketone for fuel (i.e., ketosis); ketone bodies are intermediate substrates in that metabolic sequence. This is normal when periodic, but can become a serious problem if sustained. Elevated levels of ketone bodies in the blood decrease the blood's pH, leading to DKA. On presentation at hospital, the patient in DKA is typically dehydrated and breathing rapidly and deeply. Abdominal pain is common and may be severe. The level of consciousness is typically normal until late in the process, when lethargy may progress to coma. Ketoacidosis can easily become severe enough to cause hypotension, shock, and death. Urine analysis will reveal significant levels of ketone bodies (which have exceeded their renal threshold blood levels to appear in the urine, often before other overt symptoms). Prompt, proper treatment usually results in full recovery, though death can result from inadequate or delayed treatment, or from complications (e.g., brain edema). DKA is always a medical emergency and requires medical attention. Ketoacidosis is much more common in type 1 diabetes than type 2.
Hyperglycemia Hyperosmolar State

Hyperosmolar nonketotic state (HNS) is an acute complication sharing many symptoms with DKA, but an entirely different origin and different treatment. A person with very high (usually considered to be above 300 mg/dl (16 mmol/L)) blood glucose levels, water isosmotically drawn out of cells into the blood and the kidneys eventually begin to dump glucose into the urine. This results in loss of water and an increase in blood osmolarity. If fluid is not replaced (by mouth or intravenously), the osmotic effect of high glucose levels, combined with the loss of water, will eventually lead to dehydration. The body's cells become progressively dehydrated as water is taken from them and excreted. Electrolyte imbalances are also common and are always dangerous. As with DKA, urgent medical treatment is necessary, commonly beginning with fluid volume replacement. Lethargy may ultimately progress to a coma, though this is more common in type II diabetes than type I.

Hypoglycemia

Hypoglycemia, or abnormally low blood glucose, is an acute complication of several diabetes treatments. It is rare otherwise, either in diabetic or non-diabetic patients. The patient may become agitated, sweaty, weak, and have many symptoms of sympathetic activation of the autonomic nervous system resulting in feelings akin to dread and immobilized panic. Consciousness can be altered or even lost in extreme cases, leading to coma, seizures, or even brain damage and death. In patients with diabetes, this may be caused by several factors, such as too much or incorrectly timed insulin, too much or incorrectly timed exercise (exercise decreases insulin requirements) or not enough food (specifically glucose containing carbohydrates). The variety of interactions makes cause identification difficult in many instances.

It is more accurate to note that iatrogenic hypoglycemia is typically the result of the interplay of absolute (or relative) insulin excess and compromised glucose counter regulation in type I and advanced type II diabetes. Decrements in insulin, increments in glucagon, and, absent the latter, increments in epinephrine are the primary glucose counter regulatory factors that normally prevent or (more or less rapidly) correct hypoglycemia. In insulin-deficient diabetes (exogenous) insulin levels do not decrease as glucose levels fall, and the combination of deficient glucagon and epinephrine responses causes defective glucose counter regulation.
Furthermore, reduced sympathoadrenal responses can cause hypoglycemia unawareness. The concept of hypoglycemia-associated autonomic failure (HAAF) in diabetes posits that recent incidents of hypoglycemia cause both defective glucose counter regulation and hypoglycemia unawareness. By shifting glycemic thresholds for the sympathoadrenal (including epinephrine) and the resulting neurogenic responses to lower plasma glucose concentrations, antecedent hypoglycemia leads to a vicious cycle of recurrent hypoglycemia and further impairment of glucose counter regulation.

In most cases, hypoglycemia is treated with sugary drinks or food. In severe cases, an injection of glucagon (a hormone with effects largely opposite to those of insulin) or an intravenous infusion of dextrose is used for treatment, but usually only if the person is unconscious. In any given incident, glucagon will only work once as it uses stored liver glycogen as a glucose source; in the absence of such stores, glucagon is largely ineffective. In hospitals, intravenous dextrose is often used.

**Diabetic Coma**

Diabetic coma is a medical emergency in which a person with diabetes mellitus is comatose (unconscious) because of one of the acute complications of diabetes:

1. Severe diabetic hypoglycemia
2. Diabetic ketoacidosis advanced enough to result in unconsciousness from a combination of severe hyperglycemia, dehydration and shock, and exhaustion
3. Hyperosmolar nonketotic coma in which extreme hyperglycemia and dehydration alone are sufficient to cause unconsciousness.

**Respiratory Infections**

The immune response is impaired in individuals with diabetes mellitus. Cellular studies have shown that hyperglycemia both reduces the function of immune cells and increases inflammation. The vascular effects of diabetes also tend to alter lung function, all of which leads to an increase in susceptibility to respiratory infections such as pneumonia and influenza among individuals with diabetes. Several studies also show diabetes associated with a worse disease course and slower recovery from respiratory infections (Reid and Khardori, 2008).
**Periodontal Disease**

Diabetes is associated with periodontal disease (gum disease) (Mealey, BL, 2006 Oct) and may make diabetes more difficult to treat (Glogauer, 2011). Gum disease is frequently related to bacterial infection by organisms such as Porphyromonas gingivalis and Actinobacillus actinomycetemcomitans (Mombelli, 2012). A number of trials have found improved blood sugar levels in type II diabetics who have undergone periodontal treatment (Glogauer, 2011).

**Mechanisms of Chronic Complications**

Chronic elevation of blood glucose level leads to damage of blood vessels (angiopathy). The endothelial lining the blood vessels take in more glucose than normal, since they do not depend on insulin. They then form more surface glycoprotein than normal, and cause the basement membrane to grow thicker and weaker. In diabetes, the resulting problems are grouped under "micro vascular" (due to damage to small blood vessels) and "macro vascular" (due to damage to the arteries).

However, some research challenges the theory of hyperglycemia as the cause of diabetic complications. The fact that 40% of diabetics who carefully control their blood sugar nevertheless develop neuropathy (Rich, 2006) and that some of those with good blood sugar control still develop nephropathy, requires explanation. It has been discovered that the serum of diabetics with neuropathy is toxic to nerves even if its blood sugar content is normal. Recent research suggests that in type 1 diabetics, the continuing autoimmune disease which initially destroyed the beta cells of the pancreas may also cause retinopathy, neuropathy, and nephropathy (Eguchi, 2007).

One researcher has even suggested that retinopathy may be better treated by drugs to suppress the abnormal immune system of diabetics than by blood sugar control (Adams, 2008). The familial clustering of the degree and type of diabetic complications (Greenberg, 2007) indicates that genetics may also play a role in causing complications such as diabetic retinopathy and nephropathy (Hadjadj et al., 2008). Non-diabetic offspring of type II diabetics have been found to have increased arterial stiffness and neuropathy despite normal blood glucose levels, and elevated enzyme levels associated with diabetic renal disease have been found in non-diabetic first-degree relatives of diabetics.
Even rapid tightening of blood glucose levels has been shown to worsen rather than improve diabetic complications, though it has usually been held that complications would improve over time with more normal blood sugar, provided this could be maintained. However, one study continued for 41 months found that the initial worsening of complications from improved glucose control was not followed by the expected improvement in the complications (Sandvik, 1988) In terms of pathophysiology, studies show that the two main types of DM (DM1 and DM2) cause a change in balancing of metabolites such as carbohydrates, lipids and blood coagulation factors (Alavi et al., 2011) and subsequently bring about complications like microvascular and cardiovascular complications (Nasirbagheban, 2012).

Examples of Chronic Complications

The damage to small blood vessels leads to a microangiopathy, which can cause one or more of the following:

- Diabetic cardiomyopathy, damage to the heart, leading to diastolic dysfunction and eventually heart failure.
- Diabetic nephropathy, damage to the kidney which can lead to chronic renal failure, eventually requiring dialysis. Diabetes mellitus is the most common cause of adult kidney failure worldwide in the developed world.
- Diabetic neuropathy, abnormal and decreased sensation, usually in a 'glove and stocking' distribution starting with the feet but potentially in other nerves, later often fingers and hands. When combined with damaged blood vessels this can lead to diabetic foot. Other forms of diabetic neuropathy may present as mononeuritis or autonomic neuropathy. Diabetic amyotrophy is muscle weakness due to neuropathy.
- Diabetic retinopathy, growth of friable and poor-quality new blood vessels in the retina as well as macular edema (swelling of the macula), which can lead to severe vision loss or blindness. Retinal damage (from microangiopathy) makes it the most common cause of blindness among non-elderly adults in the US.

Macrovascular disease leads to cardiovascular disease, to which accelerated atherosclerosis is a contributor:

- Coronary artery disease, leading to angina or myocardial infarction ("heart attack")
- Diabetic myonecrosis ('muscle wasting')
Peripheral vascular disease, which contributes to intermittent claudicating (exertion-related leg and foot pain) as well as diabetic foot.

Stroke (mainly the ischemic type)

Diabetic foot, often due to a combination of sensory neuropathy (numbness or insensitivity) and vascular damage, increases rates of skin (diabetic foot ulcers) and infection and, in serious cases, necrosis and gangrene. It is why diabetics are prone to leg and foot infections and why it takes longer for them to heal from leg and foot wounds. It is the most common cause of non-traumatic adult amputation, usually of toes and or feet, in the developed world.

Carotid artery stenosis does not occur more often in diabetes, and there appears to be a lower prevalence of abdominal aortic aneurysm. However, diabetes does cause higher morbidity, mortality and operative risks with these conditions (Sumpio, 2006).

Diabetic encephalopathy is the increased cognitive decline and risk of dementia— including (but not limited to) the Alzheimer's type—observed in diabetes. Various mechanisms are proposed, including alterations to the vascular supply of the brain and the interaction of insulin with the brain itself (Mailloux, 2007).

In the developed world, diabetes is the most significant cause of adult blindness in the non-elderly and the leading cause of non-traumatic amputation in adults, and diabetic nephropathy is the main illness requiring renal dialysis in the United States (Mailloux, 2007).

Risk Factors

Indians develop diabetes at a very young age, at least 10 to 15 years earlier than the western population. An early occurrence of diabetes gives ample time for development of the chronic complications of diabetes. The incidence of diabetes increases with age. In India, since the life span has increased more number of people with diabetes is being detected. The following are the important risk factors contributing for diabetes.

- Hereditary factor
- Obesity
- Aging
- Sedentary life

Pancreas

The pancreas was first identified or western civilization by Herophilus (335-280 BC), a Greek anatomist and surgeon. Only a few hundred years later, Rufus of Ephesus, another Greek anatomist, gave the pancreas its name. The term "pancreas" is derived
from the Greek ("all", "whole"), and ("flesh") – it is presumed because of its fleshy consistency.

The pancreas is a dual-function gland, having features of both endocrine and exocrine glands. The part of the pancreas with endocrine function is made up of approximately a million cell clusters called islets of Langerhans. Four main cell types exist in the islets. They are relatively difficult to distinguish using standard staining techniques, but they can be classified by their secretion: α cells secrete glucagon (increase glucose in blood), β cells secrete insulin (decrease glucose in blood), delta cells secrete somatostatin (regulates/stops α and β cells), and PP cells or gamma cells, secrete pancreatic polypeptide.

![Diagram of Pancreas](image)

**Fig. 2: L.S of Pancreas**

The islets are a compact collection of endocrine cells arranged in clusters and cords and are crisscrossed by a dense network of capillaries. The capillaries of the islets are lined by layers of endocrine cells in direct contact with vessels, and most endocrine cells are in direct contact with blood vessels, either by cytoplasm processes or by direct apposition the islets are "busily manufacturing their hormone and generally disregarding the pancreatic cells all around them, as though they were located in some completely different part of the body." The islet of Langerhans plays an imperative role in glucose metabolism and regulation of blood glucose concentration.
The pancreas as an exocrine gland helps out the digestive system. It secretes pancreatic fluid that contains digestive enzymes that pass to the small intestine. These enzymes help to further break down the carbohydrates, proteins, and lipids (fats) in the chyme.

In humans, the secretory activity of the pancreas is regulated directly via the effect of hormones in the blood on the islets of Langerhans and indirectly through the effect of the autonomic nervous system on the blood flow.

**Sympathetic (Adrenergic)**

- $\alpha_2$: Decreases secretion from beta cells, increases secretion from alpha cells,
- $\beta_2$: Increases secretion from beta cells
- $M_3$: Increases stimulation of alpha cells and beta cells

**Insulin Structure**

The symptoms of diabetes were accurately described by the ancient Egyptians, Hindus, Chinese and Greeks. A firm connection between the pancreas and diabetes was made in 1889, when the German researcher Dr Minowski removed the pancreas from dogs and found that they became diabetic.

In 1921, two Canadians, Frederick Banting and Charles Best, tested pancreatic extracts on de-pancreatised dogs and discovered the active ingredient – insulin.

In 1958, Frederick Sanger was awarded his first Nobel Prize for determining the sequence of the amino acids that make up insulin. This marked the first time that a protein had the order of its amino acids (the primary sequence) determined.

Insulin is composed of two chains of amino acids named chain A (21 amino acids) and chain B (30 amino acids) that are linked together by two disulfide bridges. There is a 3rd disulfide bridge within the A chain that links the 6th and 11th residues of the A chain together.

In most species, the length and amino acid compositions of chains A and B are similar, and the positions of the three disulfide bonds are highly conserved. For this reason, pig insulin can be used to replace deficient human insulin levels in diabetes patients. Today, porcine insulin has largely been replaced by the mass production of human proinsulin by bacteria (recombinant insulin).
Insulin molecules have a tendency to form dimers in solution, and in the presence of zinc ions, insulin dimers associate into hexamers. Whereas monomers of insulin readily diffuse through the blood and have a rapid effect, hexamers diffuse slowly and have a delayed onset of action. In the design of recombinant insulin, the structure of insulin can be modified in a way that reduces the tendency of the insulin molecule to form dimers and hexamers but that does not interrupt binding to the insulin receptor. In this way, a range of preparations of insulin is made, varying from short acting to long acting.

The 3-dimensional structure of insulin has been studied in great detail, and has provided extremely valuable information regarding its function. As previously mentioned, insulin was first crystallized in rhombohedral form in 1926 (36); and almost 10 years later Scott elucidated the importance of zinc, and other divalent cations, in crystallization (140,141). In 1969 the structure of hexameric 2-Zn insulin was determined by Dorothy Hodgkin and her coworkers using X-ray methods (65,142); and was later refined to 1.5 Å (143). Currently there are several crystal forms of insulin that have been solved, all of which display a general similarity to the initial 2-Zn insulin hexamer.

The 2-Zn insulin hexamer (MW~36000) consists of six molecules of insulin (MW~6000) arranged as three dimeric units which possesses a threefold symmetry axis (144). The dimers (MW~12000) possess a pseudo two-fold symmetry axis which is perpendicular to the three fold axis of rotation. Although each monomer of the dimers has the same peptide backbone structure they are not identical in the arrangement of certain side chains, breaking the perfect two fold symmetry. The most obvious difference is that the side chain of PheB25 is folded in towards the hydrophobic core of its respective monomer in molecule I, and out away from the monomer in molecule II (). Two Zn2+ atoms are aligned with one 8 Å above and one 8 Å below the dimer pseudo two-fold axis of rotation, lying on the three fold symmetry axis of the hexamer. Each ion is octahedrally coordinated by three HisB10 residues and three water molecules.
The 2-Zn insulin structure has provided a great deal of information about the hydrophobic, solvent exposed and potential binding surfaces of insulin. Several 2D NMR solution structures of the insulin hexamer (145), dimer (146), and monomer (147,148) have recently been elucidated. Many additional X-ray structures of insulin (148,149), insulin derivatives and insulin of other species, such as the Atlantic hagfish, Myxine glutinosa [HisB10 -> AspB10 substitution preventing Zn-binding and hexamerization] have also been examined (150,150). In most of these instances the insulin, or derivative, maintains an overall tertiary structure that corresponds well with the 2-Zn structure. For this reason the 2-Zn insulin hexamer is often used as the prototypic insulin structure, and this structure was assumed to represent the active conformation of the hormone.

Insulin exists primarily as a monomer at low concentrations (~10^-6 M) and forms dimers at higher concentrations at neutral pH (152,153). At high concentrations and in the presence of zinc ions insulin aggregates further to form hexameric complexes (65,153,154). Here we shall begin with a discussion of the insulin monomer, which is the active state of the molecule in plasma, and subsequently discuss higher aggregates of insulin.
Insulin Synthesis

The insulin-making cells of the body are called beta cells, and they are found in the pancreas gland. These cells clump together to form the "islets of Langerhans", named for the German medical student who described them. The synthesis of insulin begins at the translation of the insulin gene, which resides on chromosome 11. During translation, two introns are spliced out of the mRNA product, which encodes a protein of 110 amino acids in length. This primary translation product is called preproinsulin and is inactive. It contains a signal peptide of 24 amino acids in length, which is required for the protein to cross the cell membrane.

Once the preproinsulin reaches the endoplasmic reticulum, a protease cleaves off the signal peptide to create proinsulin. Proinsulin consists of three domains: an amino-terminal B chain, a carboxyl-terminal a chain, and a connecting peptide in the middle known as the C-peptide.

Within the endoplasmic reticulum, proinsulin is exposed to several specific peptidases that remove the C-peptide and generate the mature and active form of insulin. In the Golgi apparatus, insulin and free C-peptide are packaged into secretory granules, which accumulate in the cytoplasm of the beta cells. Exocytosis of the granules is triggered by the entry of glucose into the beta cells. The secretion of insulin has a broad impact on metabolism.

Insulin Secretion

Fig. 4: Diabetes and abnormalities in glucose-stimulated insulin secretion.
- Glucose is transported into the beta cell by type 2 glucose transporters (GLUT2). Once inside, the first step in glucose metabolism is the phosphorylation of glucose to produce glucose-6-phosphate. This step is catalyzed by glucokinase—it is the rate-limiting step in glycolysis, and it effectively traps glucose inside the cell.
- As glucose metabolism proceeds, ATP is produced in the mitochondria.
- Glucose enters the beta cells through the glucose transporter GLUT2.
- Glucose goes into glycolysis and the respiratory cycle where multiple high-energy ATP molecules are produced by oxidation.
- Dependent on the ATP:ADP ratio, and hence blood glucose levels, the ATP-dependent potassium channels ($K^+$) close and the cell membrane depolarizes.
- On depolarization, voltage controlled calcium channels ($Ca^{2+}$) open and calcium flows into the cells.
- An increased calcium level causes activation of phospholipase C, which cleaves the membrane phospholipid phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-triphosphate and diacylglycerol.
- Inositol 1, 4, 5-triphosphate (IP3) binds to receptor proteins in the membrane of endoplasmic reticulum (ER). This allows the release of $Ca^{2+}$ from the ER via IP3 gated channels, and further raises the cell concentration of calcium.
- Significantly increased amounts of calcium in the cells causes release of previously synthesized insulin, which has been stored in secretory vesicles.
- This is the main mechanism for release of insulin. In addition some insulin release takes place generally with food intake, not just glucose or carbohydrate intake, and the beta cells are also somewhat influenced by the autonomic nervous system. The signaling mechanisms controlling these linkages are not fully understood.
- Other substances known to stimulate insulin release include amino acids from ingested proteins, acetylcholine released from vagus nerve endings (parasympathetic nervous system), gastrointestinal hormones released by enteroendocrine cells of intestinal mucosa and glucose-dependent insulinotropic peptide (GIP). Three amino acids (alanine, glycine and arginine) act similarly to glucose by altering the beta cell's membrane potential. Acetylcholine triggers insulin release through phospholipase C, while the last acts through the mechanism of adenylate cyclase.
When the glucose level comes down to the usual physiologic value, insulin release from the beta cells slows or stops. If blood glucose levels drop lower than this, especially to dangerously low levels, release of hyperglycemic hormones (most prominently glucagon from Islet of Langerhan's alpha cells) forces release of glucose into the blood from cellular stores, primarily liver cell stores of glycogen. By increasing blood glucose, the hyperglycemic hormones prevent or correct life-threatening hypoglycemia. Release of insulin is strongly inhibited by the stress hormone norepinephrine (noradrenaline), which leads to increased blood glucose levels during stress.

**Insulin Receptor**

The insulin receptor is composed of two extracellular α subunits and two transmembrane β subunits linked together by disulphide bonds (Figure). Binding of insulin to the subunit induces a conformational change resulting in the autophosphorylation of a number of tyrosine residues present in the β subunit (Van Obberghen et al., 2001). These residues are recognised by phoshotyrosine-binding (PTB) domains of adaptor proteins such as members of the insulin receptor substrate family (IRS) (Saltiel and Kahn 2001; Lizcano and Alessi 2002). Receptor activation leads to the phosphorylation of key tyrosine residues on IRS proteins, some of which are recognised by the Src homology 2 (SH2) domain of the p85 regulatory subunit of PI 3-kinase (a lipid kinase).

The catalytic subunit of PI 3-kinase, p110, then phosphorylates phosphatidylinositol (4, 5) bisphosphate (PtdIns(4,5)P₂) leading to the formation of Ptd(3,4,5)P₃. A key downstream effector of Ptd(3,4,5)P₃ is AKT (otherwise known as PKB), which is recruited to the plasma membrane. Activation of AKT also requires the protein kinase 3-phosphoinositide-dependent protein kinase-1 (PDK1), which in combination with an as yet unidentified kinase leads to the phosphorylation of AKT (Figure 2). Once active, AKT enters the cytoplasm where it leads to the phosphorylation and inactivation of glycogen synthase kinase 3 (GSK3) (Figure). A major substrate of GSK3 is glycogen synthase, an enzyme that catalyses the final step in glycogen synthesis. Phosphorylation of glycogen synthase by GSK3 inhibits glycogen synthesis.
In 1991 Baynes underlined the role of oxidative stress in the evolution and progression of diabetic complications (Baynes, 1991). Oxidative stress, an imbalance between the generation of reactive oxygen species and antioxidant defense capacity of the body, is closely associated with aging and a number of diseases including cancer, cardiovascular diseases, diabetes and diabetic complications. Several mechanisms may cause oxidative insult in diabetes, although their exact contributions are not entirely clear. Accumulating evidence points to many interrelated mechanisms that increase production of reactive oxygen and nitrogen species or decrease antioxidant protection in diabetic patients.

A close relationship has been demonstrated between oxidative stress and diabetic microangiopathy, whereas the effect of the altered redox balance on endothelial function may contribute to the onset of diabetic macroangiopathy (Kuyvenhoven and Meinders, 1999). Several experimental data support the role of hyperglycemia-induced oxidative stress in the development and progression of diabetic nephropathy (Ha and Kim, 1999). Mesangial cells cultured under high levels of glucose show an increase in lipid peroxidation and synthesise a greater amount of extracellular matrix proteins (Catherwood et al., 2002). This effect is prevented by antioxidants (Catherwood et al., 2002). The protective effect of antioxidants on diabetic nephropathy has been also
observed in diabetic animals (Agardh et al., 2002; Ueno et al., 2002; Obrosova et al., 2003).

The oxidative stress is strictly influenced by glycometabolic control either in type-I (Ceriello et al., 1991) or type-II diabetics (Aydin et al., 2001). In type-II diabetics, if glycaemic control improves, the oxidative stress parameters, such as thiobarbituric acid reactive substances (TBARS), partially decrease; the same trend seems to occur for the NO$_2^\cdot$/NO$_3^\cdot$ ratio and cyclic guanosine monophosphate content. The latter should be considered a marker of endothelial dysfunction (Aydin et al., 2001). Others (Oranje et al., 1999) found, after improving the glycaemic control of type-II diabetics using insulin treatment, a decrease in conjugated dienes production rate, but no decrease in TBARS or LDL oxidation. The results of this research have been confirmed by others, (Seghrouchni et al., 2002) who demonstrated that insulin treatment nearly corrects the oxidative stress in type-I diabetics but only improves it in type- II diabetics. Because the period of insulin treatment and the HbA$_1c$ values were similar, the authors suggested the existence of metabolic differences between the two types of diabetes.

**Oxidative Stress and its Complications in Diabetes Mellitus**

Diabetes mellitus is a widespread disease with a great social impact. The quality of life and the life span of the patients with the disease depend on its complications. Hence, there is an increased interest in dealing with this disorder. Convincing evidences of the role of free radicals and oxidative stress in the pathogenesis and complications of diabetes mellitus have been established over time. It was shown that the patients were put under increasing oxidative stress in conjunction with different biochemical changes that lead to endothelial dysfunction. One of the most important is the inactivation of nitric oxide, which is key to maintaining vascular tonus. These findings underscore their importance as prognostic markers in this disease.

It has been established that oxidative stress lies at the root of a number of pathological processes and diseases such as cancer, atherosclerosis, rheumatic arthritis, haematological and neurodegenerative disorders are not exempt with more making the list among which is diabetes mellitus. To understand the essence of aetiopathogenic mechanisms, which are at the root of diabetic complications development is an essential challenge to modern medical science and practice. Nowadays diabetic micro-and macroangiopathy are considered to be polyetiological multifactorial diseases where
persistent hyperglycemia plays the leading part. On the other hand it contributes to the origin of oxidative stress. Along with the others, an endogenous and exogenous factor takes a considerable place in diabetes pathogenesis. Hence, the patients are exposed to continuously increasing oxidative stress caused by the prolonged hyperglycemia and conditioned by different pathophysiological processes.

**Free Radicals**

Free radicals are very reactive chemical species, can cause oxidation injury to the living beings by attacking the macromolecules like lipids, carbohydrates, proteins and nucleic acids. Free radical damage to LDL cholesterol leads to atherosclerosis, so antioxidants have the potential to protect against cardiovascular disease. Similarly, free radicals have been implicated in cancer, Alzheimer's disease, inflammatory diseases, ischemic-reperfusion injury and a myriad of other disease conditions against which antioxidants may be of benefit.

Most of free radicals in biological systems are derivatives of oxygen ("Reactive Oxygen Species", ROS), but there are also derivatives of nitrogen ("Reactive Nitrogen Species", RNS). Conditions of high prooxidant activity due to free radicals are often described by the phrase oxidative stress, however, the most reactive and damaging free radicals are the hydroxyl radical (especially) and the peroxynitrite radical.

Although mitochondria are the major source of free radicals, there are numerous other sources. The inflammatory cytokine Tumor Necrosis Factor-alpha (TNF-α) stimulates free radical production by mitochondria. Ultraviolet light (UV) produces free radicals. Free radicals are released from white blood cells (neutrophils) associated with inflammation. Neutrophils use oxidative free radicals (superoxide, hydrogen peroxide, and hydroxyl) to kill bacteria. The lysosomal enzyme myeloperoxidase catalyzes the production of bacteriocidal hypochlorite from hydrogen peroxide and chloride ions. Free radicals are generated by eicosanoids from arachidonic acid during ischemia-reperfusion injuries. During reperfusion the endothelial enzyme xanthine oxidase converts oxygen to superoxide, which can react with nitric oxide to produce peroxynitrite. Free radicals from tobacco smoke and air pollution can cause oxidative damage to lungs, blood vessels and other body tissues.

Under normal physiological conditions, there is a critical balance in the generation of oxygen free radicals and antioxidant defense systems used by organisms to deactivate
and protect themselves against free radical toxicity (Sies 1991; Halliwell and Whiteman, 2004). Impairment in the oxidant/antioxidant equilibrium creates a condition known as oxidative stress. Oxidative stress is known to be a component of molecular and cellular tissue damage mechanisms in a wide spectrum of human diseases (Halliwell and Gutteridge 1999; Isabella Dalle-Donne et al., 2006).

Diabetes is associated with a number of metabolic alterations and principal among these is hyperglycemia. The precise mechanism by which hyperglycemia may contribute to the development of coronary heart disease (CHD) is a matter of some controversy. Known sequelae of hyperglycemia such as cellular damage, increased extra cellular matrix production and vascular dysfunction have all been implicated in the pathogenesis of vascular disease in type-I and type-II diabetes (Halliwell and Gutteridge, 1999; Isabella Dalle-Donne et al., 2006; Guillermo Zalba et al., 2006).

Mechanisms involved in the increased oxidative stress in diabetes include not only oxygen free radical generation due to nonenzymatic glycosylation (glycation), autooxidation of glycation products, but also changes in the tissue content and activity of antioxidant defense systems. Increased levels of the products of oxidative damage to lipids have been detected in serum of diabetic patients, and their presence correlates with the development of complications (Maritim et al., 2003; Guillermo Zalba et al., 2006; Liu Shang et al., 2006). A variety of natural antioxidants exist to scavenge oxygen free radicals and prevent oxidative damage to biological membranes. One group of these antioxidants is enzymatic (intracellular), which include super oxide dismutase, glutathione peroxidase and catalase. In addition to enzymatic antioxidants, the major natural antioxidants, most of them derived from natural sources by dietary intake are vitamin A, vitamin C and vitamin E and carotenoids. Also, numerous small molecules are synthesized or produced within the body that has antioxidant capacity (ex:-glutathione and uric acid) (Halliwell and Gutteridge, 1999; Maritim et al., 2003; Heistad Donald, 2005; Engler et al., 2003). There are several studies evaluated the free radical induced lipid peroxidation and the antioxidants in diabetic patients. Many of these studies assessed individual antioxidants that act cooperatively in vivo to provide greater protection to the organism against free radical damage than could be provided by any single antioxidant acting alone. Controversial reports have been reported concerning the antioxidant status in diabetic patients (Heart Protection Study Collaborative Group-2002; Ashour et al., 1999). Protein oxidation, in contrast to lipid peroxidation, does not have the
features of chain reactions. The plasma proteins destructed by peroxidation have a quite long period. Therefore, the evaluation of protein oxidation (PCG) in plasma is a respected marker of free radical intensity. There are only a few reports regarding the protein oxidation in various other pathogenic conditions and no reports are available for that processes along with antioxidants in type I diabetic patients.

**Table 2: Different types of Free Radicals and Their Defence System**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Type of free radical or oxidants</th>
<th>Defence systems</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Superoxide anion ($O_2^{\cdot-}$)</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>2.</td>
<td>Hydroxide radical (OH)</td>
<td>(SOD), Mn-SOD, Cu, Zn-SOD</td>
</tr>
<tr>
<td>3.</td>
<td>Peroxy radical (ROO)</td>
<td>Tocopherols, Ubiquinone</td>
</tr>
<tr>
<td>4.</td>
<td>Singlet oxygen (O$_2$)</td>
<td>Carotenoids</td>
</tr>
<tr>
<td>5.</td>
<td>Hydrogen peroxide (H$_2$O$_2$)</td>
<td>Catalase, Se-glutathione peroxidase (GPx)</td>
</tr>
<tr>
<td>6.</td>
<td>Hydro peroxides (ROO)</td>
<td>Se-glutathione peroxide (GPx),</td>
</tr>
<tr>
<td>7.</td>
<td>Transition metals (Fe$^{2+}$, Cu$^{++}$)</td>
<td>Glutathione Reductase (GR chelators)</td>
</tr>
</tbody>
</table>

**Reactive Oxygen Species**

Reactive oxygen species (ROS) are ions or very small molecules that include oxygen ions, free radicals, and peroxides, both inorganic and organic. They are highly reactive due to the presence of unpaired valence shell electrons. ROS form as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling. However, during times of environmental stress (such as for example, UV or heat exposure) ROS levels can increase dramatically, which can result in significant damage to cell structures. This cumulates into a situation known as oxidative stress. They are also generated by exogenous sources such as ionizing radiation.

**Free Radical Damage**

Cells are normally able to defend themselves against ROS damage through the use of enzymes such as superoxide dismutases, catalases, glutathione peroxidases and peroxiredoxins. Small molecule antioxidants such as ascorbic acid (vitamin C), tocopherol (vitamin E), uric acid, and glutathione also play important roles as cellular antioxidants. Similarly, polyphenol antioxidants assist in preventing ROS damage by scavenging free radicals. In contrast, the antioxidant ability of the extracellular space is relatively less—e.g.: the most important plasma antioxidant in humans is probably uric acid.
In aerobic organisms the energy needed to fuel biological functions is produced in the mitochondria via the electron transport chain. In addition to energy, reactive oxygen species (ROS) are produced which have the potential to cause cellular damage. ROS can damage DNA, RNA, and proteins which theoretically contribute to the physiology of ageing.

ROS are produced as a normal product of cellular metabolism. In particular, one major contributor to oxidative damage is hydrogen peroxide ($\text{H}_2\text{O}_2$) which is converted from superoxide that leaks from the mitochondria. Within the cell there is catalase and superoxide dismutase that help to minimize the damaging effects of hydrogen peroxide by converting it into oxygen and water, benign molecules, however this conversion is not 100% efficient, and residual peroxides persist in the cell. While ROS are produced as a product of normal cellular functioning, excessive amounts can cause deleterious effects.

**Pathology of Excessive ROS Levels**

ROS have been implicated in many other major diseases that plague humans. A partial listing of these conditions (Knight, 1998; Kehrer, 1993) includes.

- The toxic effects of $\text{O}_2$ itself, such as the oxidation of lipids and proteins, generation of mutations in the DNA, and destruction of cell membranes,
- Cardiovascular diseases,
- Atherosclerosis,
- Various types of cancer,
- Diabetes,
- Neurodegenerative diseases including Parkinson's diseases and Alzheimer's disease,
- Toxicity of heavy metals, (ex:- iron)
- Radiation injury,
- Vitamin deficiency,
- Toxicity of certain medications,
- Toxic effects of tobacco smoke,
- Emphysema,
- Cataracts.
Chemistry of Biological Oxidation

When oxygen is partially reduced it becomes activated and reacts readily with a variety of bio-molecules. This partial reduction occurs in one-electron step, by addition of one, two and four electrons to O₂, which leads to successive formation of reactive oxygen metabolites (ROMs). The most common types of these reactive oxygen species (ROS) are super oxide radical (O₂⁻), hydrogen peroxide (H₂O₂), singlet oxygen (¹O₂) and the hydroxyl (HO⁻) radical, peroxide ion (HO₂).

Super oxide (O₂⁻)

Super oxide anion is the first reduction product of oxygen (O₂). It can be produced either by the univalent reduction of O₂ or by the univalent oxidation of H₂O₂. However, super oxide is not particularly reactive in biological system and does not by itself cause much oxidative damage. It is a precursor to other oxidizing agents, including singlet oxygen, peroxynitrite and other highly reactive molecules. The biological toxicity of super oxide is due to its capacity to inactivate iron-sulfur cluster containing enzymes (which are critical in a wide variety of metabolic pathways), thereby liberating free iron in the cell, which can undergo Fenton chemistry and generate the highly reactive hydroxyl radical. In its HO₂ form, super oxide can also initiate lipid peroxidation of polyunsaturated fatty acids. It also reacts with carbonyl compounds and halogenated carbons to create toxic peroxy radicals. Super oxide can also react with nitric oxide (NO) to form ONOO⁻. As such, super oxide is one of the main causes of oxidative stress. Super oxide donates one electron to reduce the metal ions that acts as the catalyst to convert hydrogen peroxide into hydroxyl radical (OH⁻).

\[ O_2^- + Fe^{3+} \rightarrow 3O_2 + Fe^{2+} \]

The reduced metal (ferrous ion or Fe²⁺) then catalyzes the breaking of the hydrogen-oxygen bond of hydrogen peroxide to produce a hydroxyl radical (°OH) and a hydroxyl ion (OH⁻).

\[ Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^° + OH^- \]

Super oxide can react with the hydroxyl radical to form singlet oxygen (O₂) which is not a radical but reactive nonetheless.

\[ O_2^- + OH \rightarrow °O_2 + OH \]
**Hydroxyl Radical (OH)**

The hydroxyl radical, \(^{\cdot}\)OH, is the neutral form of the hydroxide ion. Hydroxyl radicals are highly reactive and consequently short lived; however, they form an important part of radical chemistry. Most notably hydroxyl radicals are produced from the decomposition of hydroperoxides (ROOH). Hydrogen peroxide in the presence of metal ions (Cu\(^{+}\)/Fe\(^{2+}\)), is converted to a hydroxyl radical (\(^{\cdot}\)OH) and hydroxide ion (OH\(^{-}\)). The metal ion is required for the breaking of the oxygen-oxygen bond of peroxide. This reaction is called the "**Fenton Reaction**" and was discovered over a hundred years ago. It is important in biological systems because most cells have some level of iron, copper, of other metals which can catalyze this reaction.

\[
H:O \xrightarrow{\text{Metal ion}} \text{O:H hydrogen peroxide}
\]

\[
\downarrow
\]

\[
\text{HO}^{\cdot} \text{hydroxyl radical}
\]

And

\[
\text{HO}^{-} \text{hydroxide ion}
\]

This reaction also be written

\[
H_2O_2 + Cu^{+} / Fe^{2+} \rightarrow {^{\cdot}OH} + OH^{-} + Cu^{2+}/Fe^{3+}
\]

A hydroxyl radical can also react with superoxide to produce singlet oxygen and a hydroxide ion.

\[
\text{HO}^{\cdot} + O_2^{-} \rightarrow {^{\cdot}O_2} + OH^{-}
\]

The hydroxyl radical has a very short \textit{in vivo} half-life of approx. \(10^{-9}\) s and high reactivity. This makes it a very dangerous compound to the organism. Unlike super oxide, which can be detoxified by super oxide dismutase, the hydroxyl radical cannot be eliminated by an enzymatic reaction, as this would require its diffusion to the enzyme's active site. As diffusion is slower than the half-life of the molecule, it will react with any oxidizable compound in its vicinity. It can damage virtually all types of macromolecules: carbohydrates, nucleic acids (mutations), lipids (lipid peroxidation) and amino acids (conversion of Phe to m-Tyrosine and o-Tyrosine). The only means to protect important cellular structures is the use of antioxidants such as glutathione and effective repair systems.
Hydrogen Peroxide (H$_2$O$_2$)

Hydrogen peroxide is the most stable reactive oxygen metabolite (ROMs). This is to say that it is the least reactive and the most readily detected. H$_2$O$_2$ may be generated directly by divalent reduction of O$_2$ or indirectly by univalent reduction of super oxide anion. H$_2$O$_2$ is the primary product of the reduction of O$_2$ by numerous oxidases such as xanthine oxidase (XO), uricase, localized in peroxisomes (Ray and Husain, 2002). Hydrogen peroxide can be generated from the two electron reduction of oxygen. In biological systems hydrogen peroxide is generated by the production of super oxide: two super oxide molecules can react together to form hydrogen peroxide and oxygen.

$$2O_2 + 2H \rightarrow H_2O_2 + O_2$$

The above reaction is called dismutation reaction as the radical reactants produce nonradical products.

Although hydrogen peroxide (H$_2$O$_2$) is a non-radical form of reactive oxygen species (ROS) and only possesses moderate oxidant reactivity, it is probably more harmful than super-oxide anion (O$_2^-$), because H$_2$O$_2$ can easily diffuse across plasma membrane, enter the inner compartments of cell and can directly damage DNA, lipids, and other macromolecules causing oxidative injury to the cell. When not metabolized, H$_2$O$_2$ can react with partially reduced transition metals such as Fe$^{2+}$ or Cu$^{+}$ resulting in the generation of the extremely reactive hydroxyl radical (•OH) that will lead to the propagation of the oxidative damage to the cell (Sandstrom, 1991; Halliwell and Gutteridge, 1989).

Singlet Oxygen (•O$_2$)

It is a nonradical (does not have an unpaired electron) reactive oxygen species often associated with oxygen free radicals that has strong oxidizing activity. Singlet oxygen (•O$_2$) is an electronically excited and mutagenic form of oxygen. It is generated by input of energy like radiation, but can also be generated enzymatically by the action of peroxidases or lipoxigenases or by the reaction of hydrogen peroxide with hypochlorite or peroxynitrite. They are also generated in biological systems in a number of pigment reactions including chlorophylls, retinal and flavins when they are illuminated in the presence of oxygen.
Like many other reactive species, this can be harmful at higher concentrations and at low levels may act as signaling molecules. Due to its relatively long-life, $^1\text{O}_2$ can travel appreciable distance in the cellular environment and is capable of damaging various biomolecules (Sies and Packer, 2000). Oxidative damage in bio-molecules mediated by $^1\text{O}_2$ is rather frequent. Lipids, proteins and DNA are all at risk (Devasagayam and Kamat, 2002).

Peroxy nitrite

Peroxynitrite is the anion with the formula ONOO$^-$. It is an unstable "valence isomer" of nitrate, NO$_3^-$, which has the same formula but a different structure. Although peroxynitrous acid is highly reactive, its conjugate base peroxynitrite is stable in basic solution (Holleman and Wiberg, 2001). It is prepared by the reaction of hydrogen peroxide with nitrite.

\[ \text{H}_2\text{O}_2 + \text{NO}_2^- \rightarrow \text{ONOO}^- + \text{H}_2\text{O} \]

Peroxynitrite is an oxidant and nitrating agent. Because of its oxidizing properties, peroxynitrite can damage a wide array of molecules in cells, including DNA and proteins. Formation of peroxynitrite \textit{in vivo} has been described to the reaction of the free radical super oxide with the free radical nitric oxide (Pacher et al., 2007).

\[ \text{O}_2^- + \text{NO} \rightarrow \text{ONOO}_2^- \]

The resultant pairing of these two free radicals results in peroxynitrite, a molecule which itself is not a free radical, but is a powerful oxidant.

Antioxidant Defense Mechanism

Antioxidant means "against oxidation". Antioxidants work to protect lipids from peroxidation by radicals. Antioxidants are effective because they are willing to give up their own electrons to free radicals. When a free radical gains the electron from an antioxidant it no longer needs to attack the cell and the chain reaction of oxidation is broken. After donating an electron an antioxidant becomes a free radical by definition. Antioxidants in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive. The human body has an elaborate antioxidant defense system. Antioxidants are manufactured within the body and can also be extracted from the food humans eat such as fruits, vegetables, seeds, nuts, meat, and
oils. There are two lines of antioxidant defense within the cell. The first line, found in the fat-soluble cellular membrane consists of vitamin E, beta-carotene, and coenzyme-Q. Of these, vitamin E is considered the most potent chain breaking antioxidant within the membrane of the cell. Inside the cell water soluble antioxidant scavengers are present. These include vitamin C, glutathione peroxidase, superoxide dismutase and catalase.

About a decade ago, Scientists from various countries signed in Saas Fee (Switzerland), a declaration on the significance of antioxidants in preventive medicine. This declaration stated that antioxidant nutrients may have major significance in the prevention of number of diseases. These include cardiovascular and cerebro-vascular diseases, some forms of cancer, and several other disorders, many of which may be age-related (Nordmann, 1994).

Animal tissues are constantly coping up with high reactive oxygen species, such as superoxide anion, hydroxyl radicals, hydrogen peroxides and other radicals generating during numerous metabolic reactions (Castillo et al., 1992; Cabre et al., 2000). The generation of small amount of free radicals appears to have an important biological function, but oxidative stress is caused by excess production of reactive oxygen species (Halliwell, 1997; Giordano, 2005). To protect the cell and organ system of the body against reactive oxygen species, mammalian cells are well equipped with the highly sophisticated and complex defense mechanisms known as antioxidant defense mechanism. Antioxidant defense systems protect cellular homeostasis from oxidative disruption by reactive molecules generated through the reduction of molecular oxygen. The efficient functionality of these mechanisms requires the concerted action of the individual systems. These defense systems also have to be in concert with the components responsible for the repair processes of oxidatively damaged molecules in order in the cell integrity (Inmaculada Bando et al., 2005).

The term antioxidant has been defined by Halliwell and Gutteridge (1989), as any substance that delays or inhibits oxidative damage to a target molecule. Antioxidant enzymes, together with the substance that is capable of either reducing ROMs or preventing their formation, form a powerful reducing buffer which affects the ability of the cell to counteract the action of oxygen metabolites. All reducing agents thereby form the protective mechanisms, which maintain the lowest possible levels of ROMs inside the cell (Helmut Sies, 1997).
Antioxidant defenses, present in all aerobic organisms include antioxidant enzymes and free radical scavengers whose function is to remove reactive oxygen species (ROS), thus protecting the functions of the cells of organisms from oxidative stress (Regoli and Principato, 1995). The sensitivity of cell to oxidants is attenuated by antioxidant defense system such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione Reductase (GR), Glutathione Peroxidase (GPx), Glutathione S transferase (GST), and Glutathione content (GSH). The antioxidant defense system maintains a relatively low rate of the reactive and harmful "OH (Ismail Celik et al., 2006).

Antioxidants

Antioxidants are molecules that can neutralize free radicals by accepting or donating an electron to eliminate the unpaired condition. Typically this means that the antioxidant molecule becomes a free radical in the process of neutralizing a free radical molecule to a non-free-radical molecule. But the antioxidant molecule will usually be a much less reactive free radical than the free radical neutralized. The antioxidant molecule may be very large (allowing it to "dilute" the unpaired electron), it may be readily neutralized by another antioxidant and/or it may have another mechanism for terminating its free radical condition.

Antioxidant Enzymes

Natural antioxidant enzymes manufactured in the body provide an important defense against free radicals. Glutathione peroxidase, glutathione reductase, catalase, thioredoxin reductase, superoxide dismutase, heme oxygenase and biliverdin reductase, are the most important antioxidant enzymes. The enzyme superoxide dismutase converts two superoxide radicals into one hydrogen peroxide and one oxygen. To eliminate hydrogen peroxide before the Fenton Reaction can create a hydroxyl radical, organism's use catalase and/or glutathione peroxidase. The brain, which is very vulnerable to free radical damage (due to high metabolic rate, high unsaturated fat in neurons, and the fact that neurons are post-mitotic), has seven times more glutathione peroxidase activity than catalase activity (Marklund, 1982). Moreover, glutathione peroxidase is found throughout the cell, whereas catalase is often restricted to peroxisomes.
Superoxide Dismutase (SOD)

SOD is the most important antioxidant enzyme because it is found virtually in all aerobic organisms. SODs are a family of metalloenzymes that converts $O_2^-$ to $H_2O_2$ according to the following reaction. The transition metal of the enzyme reacts with $O_2^-$ taking its electron. $O_2^-$ is the only known substrate for SOD (Ray and Husain, 2002; Smithe et al., 2003).

$$2H^+ + O_2^- + O_2^- \xrightarrow{SOD} H_2O_2 + O_2$$

The superoxide dismutase (SOD) catalyzes the dismutation of two superoxide radicals into hydrogen peroxide and oxygen. The hydrogen peroxide is further oxidized by other enzymes. These enzymes obey first order reaction kinetics and the forward rate constants are almost diffusion limited. These results in a steady state concentration of superoxide in tissue that varies directly with the rate of superoxide generation and inversely with the tissue concentration of scavenging enzymes (Enghild et al., 1999). In humans, three forms of superoxide dismutase are present. SOD1 is located in the cytoplasm, SOD2 in the mitochondria and SOD3 is in extra cellular. The first is a dimer (consists of two units), while the others are tetramers (four subunits). SOD1 and SOD3 contain copper and zinc, while SOD2 has manganese in its reactive centre. The genes are located on chromosomes 21, 6 and 4, respectively (21q22.1, 6q25.3 and 4pl5.3-pl5.1). Simply-stated, SOD out competes damaging reactions of superoxide, thus protecting the cell from superoxide toxicity. The reaction of superoxide with non-radicals is spin forbidden. In biological systems, this means its main reactions are with itself (dismutation) or with another biological radical such as nitric oxide (NO).

SOD is biologically necessary because superoxide reacts even faster with certain targets such as NO radical, which makes peroxynitrite. Similarly, the dismutation rate is second order with respect to initial superoxide concentration. In contrast, the reaction of superoxide with SOD is first order with respect to superoxide concentration. Superoxide is one of the main reactive oxygen species in the cell and as such, SOD serves a key antioxidant role. The physiological importance of SODs is illustrated by the severe pathologies evident in mice genetically engineered to lack these enzymes. Mice lacking SOD2 die several days after birth, amidst massive oxidative stress (Li et al., 1995). Mice
lacking SOD1 develop a wide range of pathologies, including hepatocellular carcinoma (Elchuri et al., 2005), an acceleration of age-related muscle mass loss (Muller et al., 2006), an earlier incidence of cataracts and a reduced lifespan.

**Catalase (CAT)**

Catalase was first noticed by Louis Jacques Thenard in 1811. In 1900 Oscar Loew was the first to give it the name catalase. Catalase is a heme containing redox enzyme. Although the tissue distribution of Catalase is widespread, the level of activity varies not only between tissues but within the cell itself. Catalase is present predominantly in the peroxisomes (Microbodies) of liver and kidney and also in the micro peroxisomes of other tissues. It has 240,000 molecular weight in yeast, 220,000 in human blood. Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long (Boon et al., 2007). It contains four porphyrin heme (iron) groups which allow the enzyme to react with the hydrogen peroxide. Most of the in vitro studies suggested that this antioxidant function as promotion/transformation inhibitor of carcinogenesis.

Catalase catalyzes the decomposition of hydrogen peroxide to water (H₂O) and oxygen (O₂).

\[ 2 \text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \]

H₂O₂ is a powerful oxidizing agent and is potentially damaging to cells. By preventing excessive H₂O₂ build up Catalase allows important cellular processes which produce H₂O₂ as a byproduct to take place safely.

Catalase performs a very elegant 'reshuffling' of toxic compounds, i.e. peroxidative reaction, a second family of reactions catalyzed by Catalase. Possibilities for the compound RH₂ include phenols, formic acid, formaldehyde and alcohols.

\[ \text{H}_2\text{O}_2 + \text{RH}_2 \rightarrow 2\text{H}_2\text{O} + \text{R} \]

This is the trick of taking toxins and potentially harmful H₂O₂ and recombining them to produce harmless or useful products and water.

**Glutathione peroxidase (GPx)**

Glutathione peroxidase is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to...
their corresponding alcohols and to reduce free hydrogen peroxide to water. Based on selenium (Se) dependency, GSH-Px can be divided into two forms: Se dependent GSH-Px (Se-GSH-Px) and Se- independent GSH-Px (Non-Se-GSH-Px). GSH-Px is a molecule with four selenocysteine amino acid residues. The enzyme is located in both the cytosol (70%) and mitochondria (30%) of various tissues. As the integrity of the cellular and subcellular membranes depends heavily on glutathione peroxidase, the antioxidative protective system of glutathione peroxidase itself depends heavily on the presence of selenium.

Glutathione peroxidase enzyme is a well-known first line of defense against oxidative stress, which in turn requires glutathione as a cofactor. Glutathione peroxidase is considered the major detoxification enzyme for \( \text{H}_2\text{O}_2 \). Among the many functions of glutathione; it is involved in the generation of the nucleotide precursors of DNA via the reduction of ribonucleotides to deoxyribonucleotides (Meister and Anderson, 1983).

**Glutathione Reductase (GR)**

Although glutathione reductase (GR) is not directly involved in removing ROS, it serves an important role in converting GSSG to GSH, thereby maintaining GSH-Px catalytic function and a reduced intracellular redox status (Halliwell and Gutteridge, 1989). Glutathione reductase is an ancillary enzyme to limit the amounts of ROS via its reduction of GSSG in the presence of an adequate supply of NADPH thus, the ration of GSH/GSSG is maintained at a high level so that the cell maintains the capacity to combat oxidative stress.

\[
\text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow 2\text{GSH} + \text{NADPH}
\]

**Glutathione-S-transferase (GST)**

The mammalian GST super-family comprises cytosolic dimeric isoenzymes of 45-55 kDa size which have been assigned to at least four generic classes: Alpha, Mu, Pi and Theta. (Beckett and Hayes, 1992) Glutathione-S-transferase (GST) family of enzymes comprises a long list of cytosolic, mitochondrial, and microsomal proteins which are capable of multiple reactions with a multitude of substrates, both endogenous and xenobiotic. These enzymes catalyze the conjugation of a molecule of GSH to an electrophilic or other reactive species (Jakoby and Habig, 1980; Kodavanti, 1999). This activity is useful in the detoxification of endogenous compounds such as peroxidised
lipids as well as the metabolism of xenobiotics. GSTs may also bind toxins and function as transport proteins. Because of this, an early term for GSTs was ligandin (Litwack et al., 1971). GST catalyzes the conjugation of GSH with a wide variety of organic compounds, including certain species of hydroperoxides thereby shares peroxidase activity with GSH-Px (Habig et al., 1974). Unlike GSH-Px, GST activity is not affected by selenium deficiency; however GSH concentration is critical for the enzyme's catalytic function (Ji and Leeuwenburgh, 1996).

**Medicinal Plants**

India has about 45,000 plant species and several thousands have been claimed to possess medicinal properties (Grover et al., 2002). Medicinal plants used to treat hypoglycemic or hyperglycemic conditions are of considerable interest for ethno-botanical community as they are recognized to contain valuable medicinal properties in different parts of the plant and a number of plants have shown varying degree of hypoglycemic and anti-hyperglycemic activity (Grover et al., 2002).

The active principles of many plant species are isolated for direct use as drugs, lead compounds or pharmacological agents (Fabricant and Farnsworth, 2001). Several species of medicinal plants are used in the treatment of diabetes mellitus, a disease affecting large number of people world-wide. Traditional plant medicines or herbal formulations might offer a natural key to unlock diabetic complications (Nammi et al., 2003).

**Pancreoprotective and or β-Cells Regenerative and / or Insulinogenic Effect**

Inhibition of Aldose Reductase Activity

The inhibitors of aldose reductase activity have been proved to improve the diabetic complications in experimental animals (Mears et al., 1985). Flavonoids derived from various plants (Myrica multiflora) (Yosliikawa et al., 1998) and curcumin present in Curcuma longa (Awn et al., 2002) inhibit aldose reductase activity and impart beneficial action in diabetic complications (Handelsman et al., 1981).

Regulation of Key Enzymes of Metabolic Pathways

A number of hypoglycemic agents were reported to influence key enzymes of different metabolic pathways (glycolysis, glycogenolysis, gluconeogenesis, glycogenesis etc.,) thereby controlling hyperglycemia in diabetic humans and experimentally induced diabetic animals. Some decrease the production of glucose via glycogenolysis and gluconeogenesis and increase hepatic glycogenesis (Murayya koenigii and Brossicajuncea) (Khan et al., 1995), some increase transport and oxidation of glucose via glycolysis (Argimony eupatoria) (Lewis, 1949) some increase hepatic glycolysis (Bligha sapida) (Feng et al., 1958), some increase utilization of glucose by peripheral tissues Trigonella foenum graecum (Sharma, 1986), some improve glucose tolerance (Momordica charantia) (Khanna et al., 1994) and some control gluconeogenesis like Coceinia indica, (Hussain et al., 1992) Allium sativum (Sheela et al., 1992), and Latheranthum madegaskar perivinkle (Oliver and Zahand, 1979).

Scavenging Free Radicals and for Influencing Antioxidant Enzymes

‘Trasina’ a herbal formulation exhibited antihyperglycemic activity by scavenging free radicals (Bhattacharya et al., 1997) and Bordetella pertussis, (Shanti et al., 1994) Capparis deciduas (Yadav et al., 1997), Coriandrum sativum (Chhtra et al., 1999). Curcuma longa (Hussain et al., 2002) and Trigonella foenum graecum (Ravikumar et al., 1999) were reported to possess antioxidant property.

Tiwari and Rao (Tiwari et al., 2002) described the pathways of metabolism and targets where imbalance/insufficiencies in function lead to hyperglycemia and resultant diabetic syndrome. They also pointed out that phytochemicals isolated from different traditional / medicinal plants of various or similar nature exhibit multiple activities.

Various phytochemicals /drugs /diet therapies were reported to control blood glucose levels of diabetic subjects /animal models when judiciously used in selected
cases. However, these failed to control the squeal and complications of diabetes. Even the antihyperglycemic effect exerted by those appeared to be transient. None of the available therapies are satisfactory. Hence, search for natural dietary therapeutic methods for controlling diabetes are much active as diet plays a key role in the treatment of Diabetes (Hagura, 2000).

Diabetes mellitus is the major endocrine disorder (Burke, et al., 2003) responsible for renal failure, blindness or diabetic cataract (Thylefors, 1990), poor metabolic control (Donnelly et al., 2000), increased risk of cardiovascular disease including atherosclerosis and AGE (Advanced Glycation End) products (Yokozawa and Nakagawa, 2004). Antioxidants play an important role to protect against damage by reactive oxygen species and their role in diabetes has been evaluated. Many plant extracts and products were shown to possess significant antioxidant activity (Sabu and Kuttan, 2002). Hence, a diabetes literature database of medicinal plants with abstract, plant parts, objective and a ‘disease link’ to diseases other than diabetes for each medicinal plant.

According to the World Health Organization (WHO), more than 70% of the world’s population must use traditional medicine to satisfy their principal health needs (Farnsworth et al., 1985). A great number of medicinal plants used in the control of the DM have been reported (Bailey and Day, 1989; Marles and Farnsworth, 1994). However, although these plants may represent alternatives to developing new oral hypoglycemic agents, appropriate ethnobotanical information about them is scarce, obscure and ambiguous.

There is much information about the utility and study of Mexican "anti-diabetic plants" (Martinez, 1989; Aguilar, 1994). However, the analysis and discussion of the results obtained are often confused, scattered, and complicated. No publication suitably compiles the ethnobotanical and pharmacological information that allows that experimental and clinical studies with the Mexican "anti-diabetic plants" are started and continued.
**Aloe vera Plant**

*Aloe vera* is well known for its marvelous medicinal properties. These plants are one of the richest sources of health for human beings coming from nature. It has been grown as an ornamental plant widely. Products of the plant are used in the treatment of various ailments. Various parts of the plant have different effects on the body.

**Aloe vera**

Scientific classification

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>Order</td>
<td>Asparagales</td>
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<tr>
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<td>Asphodelaceae</td>
</tr>
<tr>
<td>Genus</td>
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<td>vera</td>
</tr>
<tr>
<td>Binomial name</td>
<td>Aloe vera</td>
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<tr>
<td>Telugu</td>
<td>Kalabanda</td>
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</table>

**Aloe vera** Biochemistry

*Aloe vera* is a unique plant which is a rich source of many chemical compounds (Fig. V) and plays an important role in the international market. Chemistry of the plant revealed the presence of more than 200 different biologically active substances including vitamins, minerals, enzymes, sugars, anthraquinones or phenolic compounds, lignin, saponins, sterols, amino acids and salicylic acid (Chauhan et al., 2007).

Prof. Tom D. Rowe (1941) was probably first to take vital steps in the chemical analysis of the plant. With his efforts, *A. vera* achieved its first detailed evaluation. *Aloe vera* now reported to contain as many as 75 nutrients and 200 active compounds including sugar, anthraquinones, saponins, vitamins, enzymes, minerals, lignin, salicylic acid and amino acids (Vogler and Ernst, 1999, Dureja et al., 2005; Park and, 2006). List of all the constituents are given in Table 2.1.
Fig. 6: Components of Aloe vera

Sugars (muco-polysaccharides) derived from the mucilage layer of the plant, surrounds the inner gel. Aloe vera contains both mono and polysaccharides. Ikawa and Niemann (1951) found that the mucilage of Aloe vera consists especially of the muco-polysaccharides glucose, mannose and uronic acid. Other reports stating the presence of glucose and a polyuronide consisting of a high molecular weight glucose mannose polyose (MW upto about $2.75 \times 10^5$) and hexuronic acid along with traces of lactose, arabinose and xylose (Gjerstad, 1971). (Ritchey 1972) reports the occurrence of free amino acids and free monosaccharide. However, (Ovodova et al., 1975) reported the presence of uronic acid. Aloe vera juice, about 99.52% water, contains carbohydrates, glucose and polyuronide (Waller et al., 1978). Chemical analysis has shown that the gel contain various carbohydrate polymers notably either gluco-mannans or pectic acid along with a range of other organic and inorganic components (Grindlay and Reynolds 1986). According to (Mabusela, 1990) primary constituents are glucomannoglycan polysaccharides containing acetylated monosaccharides (70-80%). In Aloe vera, five saccharides, namely, arabinose, galactose, glucose, mannose and xylose are present (Davis 1997).

Acemannan, a complex mannose carbohydrate derived from the Aloe vera plant has an inherent viscosity (Tello et al., 1998). (Danhof, 1998) stated that the Aloe polysaccharides consist of linear chains of $\beta$-1-4 linked glucose and mannose molecules, known as gluco-mannans. Reynolds and (Dweck 1999) listed 16 different polysaccharides that have been extracted from Aloe vera leaf gel.
Anthraquinones are the phenolic compounds which are found in the sap (Fig. VI). (Stenhouse 1851) were first to identify the principle active substance of the plant and Smith (1851) named it ‘Aloin’. The crystalline glycosides known as ‘aloin’ were first prepared by T. Smith and H. Smith of Edinburgh in 1851. The major (25-40%) constituent of Aloe is the Hydroxyanthraquinoid derivatives- aloin (= barbaloin, a mixture of aloin A and B, the diastereoisomeric 10-C glucosides of Aloe-emodin anthrone) and 7-hydroxyaloain isomers. Other constituents present in minor quantities include Aloe-emodin, chrysophanol; derivatives-aloresin B (= aloesin, upto 30%) with its p-coumaryl derivative aloeresins A and C and the aglycone aloesone (Hirata and Suga 1983, Van Wyk et al., 1995 and Dagne et al., 2000).
Table 3: Constituents of *Aloe vera* (Shelton 1991, Vogler and Ernst 1999)

<table>
<thead>
<tr>
<th>Anthraquinones</th>
<th>Saccharides</th>
<th>Vitamins</th>
<th>Inorganic Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloin / Barbaloin</td>
<td>Cellulose</td>
<td>B1</td>
<td>Calcium</td>
</tr>
<tr>
<td>Isobarbaloin</td>
<td>Glucose</td>
<td>B2</td>
<td>Sodium</td>
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<tr>
<td><em>Aloe</em>-emodin</td>
<td>Mannose</td>
<td>B6</td>
<td>Chlorine</td>
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<tr>
<td>Emodin</td>
<td>L-Rhamnose</td>
<td>Choline</td>
<td>Manganese</td>
</tr>
<tr>
<td>Aloetic Acid</td>
<td>Aldopentose</td>
<td>Folic Acid</td>
<td>Zinc</td>
</tr>
<tr>
<td>Ester of Cinnamic Acid</td>
<td>Ascorbic Acid</td>
<td></td>
<td>Chromium</td>
</tr>
<tr>
<td>Anthranol</td>
<td>a-Tocopherol</td>
<td></td>
<td>Copper</td>
</tr>
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<td>Chrysophanic Acid</td>
<td>β-Carotene</td>
<td></td>
<td>Magnesium</td>
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<tr>
<td>Resistannol Anthracene</td>
<td></td>
<td></td>
<td>Iron</td>
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<tr>
<td>Ethereal oil</td>
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<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Nonessential Amino Acids</th>
<th>Essential Amino Acids</th>
<th>Miscellaneous</th>
</tr>
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<tbody>
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<td>Cyclooxygenase</td>
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<td>Lysine</td>
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<td>Catalase</td>
<td>Aspartic Acid</td>
<td>Leucine</td>
<td>β-Sitosterol</td>
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<tr>
<td>Lipase</td>
<td>Glutamic Acid</td>
<td>Isoleucine</td>
<td>Lignins</td>
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<tr>
<td>Alkaline phosphatase</td>
<td>Proline</td>
<td>Phenylalanine</td>
<td>Uric Acid</td>
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<td>Carboxypeptidase</td>
<td>Glycine</td>
<td>Methionine</td>
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<td></td>
<td>Alanine</td>
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<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td>Arachidonic Acid</td>
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</table>

The main crystalline glycoside, barbaloin, is found in all the commercial varieties. A polarographic method has been developed for estimating aloins and *Aloe*-emodins in *Aloe* by (Stone and Furman 1947). Aloin is a bitter juice that dries to a yellow powder, can be hydrolyzed to *Aloe*-emodin slightly soluble in water and organic solvents (Hay and Haynes 1956, McCarthy 1969, Hirata and Suga 1977, Budavari 1989, Shelton 1991, Van Wyk *et al.*, 1995, Viljoen *et al.*, 2001). Aloin A and B are collectively known as barbaloin and these are most outstanding members of this class (Hay and Haynes 1956, Tyler *et al.*, 1994, Rauwald 1989 and Mannitto *et al.*, 1990). (Ishii *et al.*, 1984) used HPLC and TLC to detect barbaloin content within *Aloe* plants. The barbaloin content of latex from different *Aloe* species was assessed by a number of methods and found to be between 10-25% on a dry weight basis of the latex and approximately 1% on a leaf dry

![Structures of main Anthraquinone compound of Aloe vera](image)

Fig. 7: Structures of the main Anthraquinone compound of *Aloe vera*

According to (Duke and Beckstrom-Sternberg, 1994), the flavor and extract manufacturers Association accepted level for *Aloe vera* was 5-2,000 ppm. The major leaf exudate compounds detected were aloeresin, aloesin, aloin A, aloin B, aloinoside B, aloinoside (Viljoen *et al.*, 2001). *Aloe vera* products may be considered aloin-free if maximum limit of 0.1 mg/l is not exceeded (European Council 1988). According to the International *Aloe* Science Council (IASC 2003), the maximum allowable aloin content in *Aloe*-derived material for non-medical use is 50 ppm or lower. UE and Switzerland legislation gave a limiting value for the maximum content of aloin in foodstuffs. This value is fixed at 0.1 mg/kg, expressed in total aloin content i.e. A+ B isomers. Phenolic anthraquinones were separated and characterized using TLC, HPLC and column chromatography by (Rajendran *et al.*, 2007). Thirteen phenolic components namely, aloesin, 8-C-glucosyl-7-Omethyl-(S)-aloesol, neoaloesin A, 8-O-methyl-7- hydroxyaloin
A and B, 10-hydroxyaloin A, isoaloeresin D, aloin A and B, aloeresin E, Aloe-emodin, aloenin and aloenin B in A. barbadensis and A. arborescens were separated and quantified by the HPLC method. HPLC has been used to quantitate barbaloin in Aloe capsules with an LOD of 0.02 μg (Chen et al., 2002) and for the detection of Aloe components in creams (Yamamoto et al., 1985). Aloin (or barbaloin) is known as the main laxative component of Aloe preparations, and it has generally been used as a key component for the quality control of pharmaceuticals containing Aloe (Ishii et al., 1984 and Zonta et al., 1995). The aloin content varied between 4.5 to 25%. Saponins, the glycosides having cleansing and antiseptic capability, constitute 2.91% (Wasicky and Hoehne 1951) and 3.0% (Hirata and Suga, 1983) of the leaf gel.

Aloe vera contains many vitamins including A, B₁, B₂, B₆, C, E and F excluding vit. D (Chauhan et al., 2007). Some authors also suggested the presence of vitamin B₁₂ (Cynocobalamin) in trace amount (Coats 1979, Antherton 1998 and Dureja 2005). Meadows (1980) reported 6 enzymes in the Aloe vera gel including Bradykinase, Cellulase, Carboxypeptidase, Catalase, amylase and an oxidase. Carboxypeptidase inactivates bradykinase and produces an anti-inflammatory effect (Shelton, 1991). Enzymes such as acid phosphatase, alkaline phosphatase, amylase, lactic dehydrogenase and lipase have also been reported in the gel (Hayes, 1999).

Sodium, potassium, calcium and magnesium are the predominant minerals detected in all leaf fractions, however, calcium is the main mineral detected in the rind and pulp while sodium and potassium are higher in the gel (Robson et al., 1982). (Yamaguchi et al., 1993) reported the presence of aluminium, boron, calcium, iron, magnesium, sodium, phosphorus, silicon and strontium in Aloe vera gel. (Wang and Tung, 1993) studied mineral composition of Aloe vera juice and reported that potassium and chloride concentrations appeared to be excessive for most plant products.

Lignin, a pulp like substance existing in a formation with cellulose comprising the leaf gel in Aloe vera, was first discovered in Aloe vera gel by (Wang 1993). (Gjersted and Bouchey 1969) concluded that Aloe vera juice contained the 18 amino acids out of 22 found in human body. (Waller et al., 1978) showed that Aloe barbadensis leaves contain various amino acids with highest concentration of arginine (449 μmole/100g), followed by asparagine (344 μmole/100g), glutamate (294 μmole/g), aspartate (2 37 μmole/g) and serine (224 μmole/100g). Seven of the eight essential amino acids required by human body are also present in Aloe gel (Chauhan et al., 2007).
Aloe vera and Medicinal Value

Biological activities of Aloe vera have been established by large number of studies. Because of its demand, it is cultivated in large quantities in many parts of the world (Newall et al., 1996). It has multiple constituents possessing potential biological activities (Femenia et al., 1999). The Aloe vera plants have been used worldwide due to its medicinal properties (Fig. 8).

Fig. 8: Medicinal uses of Aloe vera

Aloe vera has enjoyed a long history as a herbal remedy and is most popular herbal plant. Major value added products from Aloe are gel and juice. Plants belonging to genus Aloe particularly Aloe vera (=A. barbadensis) have been known for their medicinal properties for many centuries (Antherton 1998 and Urch 1999). According to (Wahid and Siddique 1961) the plant is used as anti-septic, germicidal, blood purifier and in chronic ulcers to stimulate healing. It is used in many products such as fresh gel, juice and other formulations of health, medical, toiletries and cosmetic purposes (Morten 1961, Blitz et al., 1963, Cera et al., 1980, Leung 1985, Genet and Van Schooten 1992, Antherton 1998, Leon 2003 and Pine 2003). List of some of the products which are commercially available are given in Table 2.2.

The Aloe vera gel has moisturizing effect by providing a sustained moist environment when applied to drying tissues that promote retention of moisture in tissues (Meadows 1980). This is the major component found in many commercial products found in preserved but otherwise untreated form (McAnalley 1988, 1990, Reynolds and Dweck 1999). Aloe vera is available in a large range of skin moisturizers, face and hand creams, cleansers, soaps, shampoos, hair tonics, shaving preparations, bath aids, make-up and fragrance preparations, baby lotions and wipes (Gallagher and Gray 2003). Aloe vera is a valuable ingredient for food, pharmaceuticals and cosmetic industries (Choo 2003, Eshun and He 2004 and Neall 2004). (Wei et al., 2004) prepared a health beverage from fresh Aloe vera leaves. The gel has been found to be useful in extending the shelf life of grapes (Valverde et al., 2005) and sweet cherries (Martinez et al., 2006).
Table 3: Commercially available *Aloe* products

<table>
<thead>
<tr>
<th>S. No</th>
<th>Preparation</th>
<th>Use</th>
<th>Manufactures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Gel</td>
<td>Skin diseases, burns, cuts, wounds, ulcers, eruptions etc</td>
<td>S.S. Life Sciences Pvt. Limited</td>
</tr>
<tr>
<td>2.</td>
<td>Shampoo</td>
<td>Prevents hair loss, antidandruff, revitalizes hair roots chemical free and suitable for all kinds of hairs</td>
<td>Besure Health Pvt. Limited, New Delhi</td>
</tr>
<tr>
<td>3.</td>
<td>Body wash</td>
<td>Hydrate and nourish the skin, protects the skin from UV Rays and others skin infections</td>
<td>Besure Health Pvt. Limited, New Delhi</td>
</tr>
<tr>
<td>4.</td>
<td>Body lotion</td>
<td>Hydrate, moisturizes and receive the skin. Repair damage cells, gives soft and healthy skin.</td>
<td>Forever living products company</td>
</tr>
<tr>
<td>5.</td>
<td>Tea</td>
<td>Low calorie. No caffeine, refreshing</td>
<td>Forever living products company</td>
</tr>
<tr>
<td>6.</td>
<td>Sunscreen</td>
<td>High SPF for more sun protection, safe and gentle, sooth, lubricate, moisturize and protects the skin again sunlight and wind</td>
<td>Forever living products company</td>
</tr>
<tr>
<td></td>
<td><strong>Product</strong></td>
<td><strong>Description</strong></td>
<td><strong>Supplier</strong></td>
</tr>
<tr>
<td>---</td>
<td>------------------------------</td>
<td>---------------------------------------------------------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>7</td>
<td><strong>Toxin charging capsules</strong></td>
<td>Improve digestion, help reducing fat</td>
<td>Yuan Tang pharmacy Group Ltd.</td>
</tr>
<tr>
<td>8</td>
<td><strong>Soothing night cream</strong></td>
<td>Moisturize, comfort and calm and soothe the skin</td>
<td>Shop online</td>
</tr>
<tr>
<td>9</td>
<td><strong>Cosmetics</strong></td>
<td>High effective for maintaining skin healthy. No chemicals are used.</td>
<td>Skin care naturals (P.) Ltd. Delhi</td>
</tr>
<tr>
<td>10</td>
<td><strong>Arthritis cream</strong></td>
<td><em>Aloe vera</em> is a powerful anti-inflammatory and an analgesic.</td>
<td>Besure Health Pvt. Limited, New Delhi</td>
</tr>
<tr>
<td>11</td>
<td><strong>Aloe vera cream</strong></td>
<td>Highly effective to protect skin from atmospheric pollution and blemishes. Give freshness to the skin.</td>
<td>Kapoor herbal products</td>
</tr>
<tr>
<td>12</td>
<td><strong>Soft gels (500MG)</strong></td>
<td>Helps in treating ulcer diseases, helps to heal external wounds, wound healing including burns</td>
<td>Nutri-force nutrition</td>
</tr>
<tr>
<td>13</td>
<td><strong>Aloe vera soap</strong></td>
<td>Helps to restore and repair skin. Most beneficial if some kind of fungal skin infection.</td>
<td>Pinnacle wellness concepts Pvt. Ltd, one of chief Aloe vera liquid soap manufacturer all over the World.</td>
</tr>
</tbody>
</table>
Cleopatra reportedly used Aloe for its cosmetic benefits (Wright 2003). Many beneficial effects of this plant have been attributed to the polysaccharides present in the pulp. The clear pulp which is also known as gel is widely used in various medical, cosmetic and neutraceutical applications (Ni et al., 2004). Studies by (Hu et al., 2005) noted that higher antioxidative activities of Aloe vera extract is due to components in its rind. Aloe vera has been used externally to treat various skin conditions such as cuts, burns and eczema (Serrano 2006). These Aloe species are currently listed in the pharmacopoeia of many countries in form of pain Aloe, extract and powder (Park and Jo 2006).

Wound Healing Properties

Aloe gel is widely used for treatment of wounds and inflammatory skin disorders. Aloe vera has a very ancient history for healing wounds and also found in folk medicine for the treatment of burns and chronic wounds (Saleem et al., 1997). Clinical investigations suggest that Aloe gel preparations accelerate wound healing (Bruneton 1995).

According to (Mackee 1938), vitamin D was the healing agent. (Zawahry et al., 1973) deduced that the active healing principle existed in the mucopolysaccharides. (Davis et al., 1989) noted that Aloe vera gel improved wound healing by increasing blood supply which increased oxygenation as a result. According to (Fulton 1990), (Chithra et al., 1998) and (Azenedo et al., 2001), Aloe accelerates the wound healing while according to (Heggers 1996) Aloe gel treatment accelerates wound contraction. (Thompson 1991) reported that topical application of the Aloe vera derived allantoin gel stimulated fibroblast activity and collagen proliferation. Study by (Heggers et al., 1993) showed that topical application of Aloe vera gel re-established vascularity of burn tissue for a guinea pig, although no specific constituents were identified. A mannose-6-phosphate component of the gel has been credited with a wound healing effect (Davis et al., 1994). (Heggers et al., 1995) found a commercial preparation of Aloe vera gel decreased the healing time of full thickness excision wounds in rats. A 1996 study reported that a high molecular weight polypeptide constituent from the gel demonstrated a healing effect on excisional wounds in rats (Heggers 1996). (Yagi et al., 1997) reported that Aloe vera gel contains a glycoprotein which has cell proliferating promoting activity. The Aloe vera gel polysaccharide acemannan was shown to activate macrophages, an effect that improved wound healing (Tizard et al., 1994 and Maxwell et al., 1996) in a rat. (Mantle et al., 2001) studied the adverse and beneficial effects of plant extracts on skin.
(Rodriguez-Bigas et al., 1988) found a commercial preparation of Aloe vera gel had better healing effects on full-thickness wounds in guinea pigs when compared with other burn wounds. Preparations of Aloe vera have been used to treat radiation dermatitis and reported results in guinea pig when Aloe vera gel was used to treat burn wounds (Kaufman et al., 1988). Aloe vera gel is widely used as a natural remedy for burns (Haller 1990). In a human study, 27 patients with partial thickness burn wounds were treated with topical Aloe gel or standard Vaseline gauze (Visuthikosol et al., 1995).

Some of the first scientific studies on the effectiveness of Aloe gel were performed during the 1930s and involved protection of the skin against radiation damage. Aloe gel was effective in the treatment for radiation injury (Fine and Samuel 1938 and Williams et al., 1996). The expressed juice of Aloe vera in the form of an ointment in Vaseline has been found to hasten healing of wounds of thermal burns and radiation injury in albino rats (Singh et al., 1973). A positive effect also was documented in mouse skin exposed to soft x-irradiation (Sato et al., 1990). An acemannan containing topical gel was demonstrated to reduce skin damage following exposure to gamma radiation in mice (Roberts and Travis 1995). (Pande et al., 1998) studied that treatment with the Aloe extract reduced radiation induced damage to germ cells and loss in body weight. It is a home remedy that can be used as a moisturizing agent and for the treatment of minor burns, skin abrasions and irritations (Tarro 1993 and Krinsky 2003). Studies shows the Aloe vera gel contain immunomodulator that prevent ultraviolet B induced damage on epidermal cell. There are reports of successful treatment of X-ray and radium burns (Mandeville 1939, Row 1941, Lusbbbaugh and Hale 1953 and Brown 1963).

Anti-diabetic Properties

(Ghannam et al., 1986) reported the anti-diabetic effect of Aloe vera in alloxan-induced mice. Extracts of Aloe increases glucose tolerance in both normal and diabetic rats (Al-Awadi and Gumaa 1987). Anti-diabetic potentials of Aloe plants have been established by various workers (Agarwal 1985, Bunyapraphatsara et al., 1996, Yongchayiudha et al., 1996, Okayar 2001, Grover et al., 2002 and Jones 2004). Oral administration of Aloe vera was helpful for lowering blood glucose in diabetic patients (Yongchayiudha et al., 1996 and Bunyapraphatsara et al., 1996) as well as for reducing blood lipid level (Nassiff et al., 1993). Significant decrease in blood-glucose level after oral administration of ethanol extract of Aloe vera (Rajasekaran et al., 2005). There are some evidences that shows Aloe vera extract may be useful in the treatment of diabetes (Boudreau et al., 2006).
Laxative Effects

*Aloe vera* latex possesses laxative properties and use of the latex to reliance constipation dates back to classic Greece with first recordings of its use in the first century A.D. (Fantus 1922). Gel is also used as a purgative. (Smith and smith 1851) are credited with the discovery of active purgative principle of the latex and for naming the crystalline substance aloin. The drug Bitter *Aloes* has been used for several thousands of years because of its purgative properties (Reynolds 1983). Anthraquinones present in *Aloe* latex function as potent stimulant laxative. Studies in rats have shown that *Aloe* latex increases intestinal water content, stimulants, mucus secretion and increases intestinal peristalsis (Ishii *et al.*, 1994). *Aloe* latex is used for its laxative effect (Cheney 1970 and Capasso *et al.*, 1998).

*Aloe vera in Dentistry*

*Aloe vera* increases rate of healing of periodontal surgery wounds (Henry 1979). (Hayes1999) discussed the beneficial effects of *Aloe vera* treatment in a case study of patient diagnosed with 'Lichen Planus', a disease that is brought about by emotional distress and caused lace-like lesions in the oral cavity and on the skin. Similar effect has been reported by (Choonhakarn *et al.*, 2007). (Uzma *et al.*, 2008) concluded from their study that *Aloe vera* gel was a safe and effective treatment for patients with vulval lichen planus, a chronic inflammatory disorder of mucosal surfaces.

Anti-microbial Properties

*Aloe vera* extracts are utilized in the development of anti-bacterial and anti-fungal products (Farnsworth 1984). Scientific studies support anti-bacterial and anti-fungal effect for substances in *Aloe vera* (Klein and Penneys 1988). Anti-microbial potentials of *Aloe* plants have been investigated by a number of workers. In 1964, Lorenzetti and associates tested leaves of *Aloe vera* against a variety of bacteria. (Heggers *et al.*, 1979) tested *Aloe vera* gel and Dermaide *Aloe* against ten bacterial strains viz.,*Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Escherichia coli*, *Serratia marcescens*, *Klebsiella sp.*, *Enterobacter sp.*, *Citrobacter sp.*, *Bacillus subtilis* and *Candida albicans*. At 90% concentration *Aloe vera* gel was effective against all the organisms but at the 70% concentration only against *S. pyogenes*. Dermaide *Aloe* was effective against all organisms at a concentration of 70%. Heck *et al.*, (1981) tested preserved *Aloe* gel extract and an unpreserved *Aloe* extract against *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. It was found that preserved *Aloe* gel extract was more effective in controlling bacterial growth than unpreserved one. (Roboson *et
al., 1982) studied the anti-bacterial effects of Aloe vera extract and found that concentration as low as 60% were bactericidal against seven of the 12 species of organisms studied. (Heggers et al., 1995) suggested that the anti-bacterial effect of the Aloe vera gel could enhance the wound healing process by eliminating the bacteria that contribute to inflammation. Aloe vera gel was shown to inhibit the growth of gram positive bacteria, Shigella flexneri and Streptococcus pyogenes (Ferro et al., 2003). The anti-microbial activity of Aloe vera juice was investigated by (Cock Ian Edwin2008) by agar disc diffusion against a panel of bacteria, fungi and yeast. Aloe vera juice showed anti-bacterial activity against only the Gram-negative bacteria A. hydrophilia and E.coli and not against any fungi or yeast tested. Similar results have been obtained by (Alemdar and Agaoglu 2009) and established the anti-microbial activity of the Aloe vera juice against Gram-positive bacteria (Mycobacterium smegmatis, Staphylococcus aureus, Enterococcus faecalis, Micrococcus luteus and Bacillus sphericus), Gram- negative bacteria (Pseudomonas aeruginosa, Klebsiella pneumonniae, E.coli, and Salmonella typhimurium) and Candida albicans in vitro. A variety of isolates from Aloe vera were shown to inhibit microbes like Staphylococcus aureus (Martinez et al., 1996, Agarry et al., 2005, Cete et al., 2005 and Kaithwas et al., 2008), Candida albicans (Stuart et al., 1997 and Agarry et al., 2005), Pseudomonas aeruginosa (Soeda et al., 1966 and Cete et al., 2005), Klebstella pneumonniae (Heggers et al., 1979 and Heck et al., 1981). Acemannan, a polysaccharide component from Aloe, has been proposed to have indirect anti-microbial activity through its ability to stimulate phagocytic leukocytes (Lawless and Allan 2000 and Pugh et al., 2001). The anti-bacterial activity of leaves is attributed to anthraquinones (Boateng 2000, Garcia-Sosa et al., 2006 and Dabai et al., 2007) and saponins (Reynolds and Dweck 1999 and Urch 1999).

Anti-fungal Activities

Fujita et al., (1978) established anti-fungal activity of leaf powder of Aloe barbadensis and A. arborescens against three strains of Trichophyton mentagrophytes. (Kawai et al., 1998) studied activity of Aloe arborescens Miller var. natalensis. (Ali et al., 1999) found that the extracts of fresh leaves of Aloe barbadensis and Aloe arborescens have anti-fungal potential against Aspergillus niger, Cladosporium herbarum and Fusarium moniliforme. Aloe vera extract have been shown to inhibit the growth of fungi that cause tinea (Sumbul Shamin et al., 2004).
Anti-viral Properties

Womble and Helderman (1988) published the first study suggesting that acemannan isolated from Aloe had an anti-viral effect on human cells. Acemannan reduced herpes simplex infection in two cultured target cell lines (Kemp et al., 1990). (Kahlon et al., 1991) tested acemannan for anti-viral activity against HIV-I. Glycerine extract of Aloe barbadensis inactivated a variety of viruses, including Herpes simplex virus (HSV-1 and HSV-2), Pseudorabies virus, varicella-Zoster virus, influenza virus, rhinovirus and adenovirus (Sydiskis et al., 1991). (Yates et al., 1992) demonstrated the anti-viral activity of acemannan in a pilot study of clinically symptomatic immuno-deficiency virus infected cats. (Zandi et al., 2007) tested the anti-viral activity of a crude hot glycerine extract of Aloe vera gel against HSV-2 replication in Vero cell line (African green monkey kidney cell line). Extracts of leaves of Aloe have been reported to have anti-viral activity by a number of workers (Andersen et al., 1991, Bernard et al., 1992, Pottage and Kessler 1995, Cohen et al., 1996, Semple et al., 2001 and Malvy et al., 2005).

Toxicity

Aloe gel has few side effects. The Handbook of Medicinal Herbs (Duke and Fulton 2002) has given Aloe the lowest ranking for toxicity. In doses of 0.6 gm a day is very unsafe for pregnant women and is likely to produce abortion (Chopra et al., 1965). (Brasher et al., 1969) mixed Aloe vera gel with prednisolve and indomethacin and incorporated it into cell maintenance medium to look for cytotoxicity in He La cells and rabbit kidney fibroblast. They found $5 \times 10^{-1}$ dilution of Aloe gel was toxic while dilutions at $10^{-1}, 10^{-2}$, and $10^{-3}$ were not. It is contraindicated in pregnancy and in individuals afflicted with hemorrhoids and can cause kidney irritation (Duke 1981). It should not be used during pregnancy except under medical supervision (Lewis and Weingold 1985). It should not used by nursing mothers (Brinker 1998). A dose of 0.5g per day could produce a strong purgative and oxytoxic properties provoking uterine contractions in females (George, 1999).

Winters et al., (1981) found Aloe vera gel was cytotoxic for human normal and tumor cells in vitro. (Danof and McAnalley 1983) tested four commercial stabilized Aloe vera gel samples and yellow Aloe sap for cytotoxicity. They discovered sap was lethal to human fibroblasts and two gel products were cytotoxic to human endothelial cells and fibroblasts. Formulations of acemannan had significant cytotoxicity against human fibroblasts (Tello et al., 1998).
There have also been several reports of Aloe gel lowering plasma glucose levels in laboratory animals and in humans (Ghannam et al., 1986 and Ajabnoor 1990). There are reports of burning sensations and development of dermatitis on the face of patients who applied gel (Hunter and Frumkin 1991). It was also reported to have genetic toxicity (Muller et al., 1996). Excessive use of Aloe extract causes diarrhoea, nephritis, gastritis and vomiting (Capasso et al., 1998 and Goodman and Gilman 1990). Hypersensitivity (Morrow et al., 1980) and allergic conditions to Aloe preparations have been reported (Ernst 2000). Besides, the first case of acute hepatitis due to ingestion of this compound was described (Rabe et al., 2005 and Yang 2010).

Other therapeutic uses

Tyler (1994) postulated that some of the beneficial effects of Aloe are a result of a carboxypeptidase that inhibit the pain-producing agent bradykinin. (Corsi et al., 1998) said that treatment with Aloe vera, controlled tumour growth and prolonged the survival of rats compared with control. Aloe species are used around the world for conditions ranging from dermatitis to cancer (Kemper and Chiou 1999).

Aloe vera plants have been used as for the treatment of hepatitis (Kim et al., 1999). Studies by (Lee et al., 2000) shown that the aqueous ethanol extract of Aloe vera powder have anti-mutagenic and anti-leukemic activities. Antioxidant effects have been studied in many studies (Lee et al., 2000 and Hu et al., 2003). A study by (Hu et al., 2003) used an assay system for free radicals to confirm the antioxidant action of Aloe vera extract.

Aloe contains more than 200 substances which are responsible for its different types of health and nutritional benefits. In Veterinary medicine, Aloe is used as drug to treat tumours in dogs and cats (Dureja et al., 2005). It is evaluated as generally safe (Vogler and Ernst 1999 and Bernardelli 2002).

Molecular Genetic Studies

Genetic characterization of Aloe vera been attempted by few workers. Adams et al., (2000) investigated the physical organization of 18S-5.8S-26S and 5S ribosomal DNA (rDNA) using fluorescent in situ hybridization (FISH) to 13 Aloe species. (Hiroko et al., 2003) used RAPD analysis for identification of three species of Aloe (Aloe vera, A. arborescence, Aloe ferox). A team of workers at CIMAP, Lucknow (Darokar et al., 2003) carried out the molecular assessment of diversity in Aloe using RAPD and AFLP analysis. (Yagi et al., 2006) compared ribosomal DNA sequence analysis of different geographically distributed Aloe vera plants with clonally regenerated plants.
Tissue Culture Studies

The majority of plants used for medicine are harvested from the wild. This result in serious problems like depletion of resources, extinction of rare species, insufficient supplies, seasonal collections, incorrect identification, and adulterations in dried materials, etc. Systematic cultivation of medicinal plants instead of collecting them from the wild minimizes many of the above problems. Cultivation of Aloe for commercial purpose has been adopted in many countries. This is exclusively a vegetatively propagated crop where young side branches are used as planting material. Single plant may produce 2-3 side shoots per year making availability of planting material in good quantity and quality, a problem. Tissue culture techniques for micro-propagation are being used profitably to overcome such problems in various crops, ornamental and horticulture plants. Tissue culture research began nearly four decades ago with the first report on production of test tube fertilization (Kanta and Maheshwari, 1963).

A number of protocols for micropropagation of Aloe plants have been developed using a variety of explants like shoot tip, axillary bud, stem cuttings etc. by various researchers. Aloe vera has been cultured by in vitro by various researchers like (Sanchez et al., 1988, Meyer and Staden 1991, Corneanu et al., 1994, Richwine et al., 1995, Abrie and Staden 2001, (Hosseini and Parsa 2007).

Hirimburegama and Gamage (1995) found that high rates of shoot proliferation were obtained from axillary and apical buds of Aloe vera cultured on MS media supplemented with 0.18 mg/l IAA+ 2.25 mg/l BA. Rooting was achieved on MS media with 0.18 mg/l IAA+ 0.226 mg/l BA for 3 weeks. (Richwine et al., 1995) reported the induction of shoot cultures of Aloe, Gasteria and Howorthia species from immature inflorescence. They used modified MS medium containing zeatin and later maintained on zeatin and BA containing medium. Gui et al., (1990) successfully regenerated the adventitious buds obtained during the stem tissue culture and organogenesis in Aloe and found that zeatin was better than (kinetin. Zhou Yu Ding et al., 1999) suggested that number of regenerated buds could be increased 8-9 times following bud splitting before culturing on growth media. For induction of buds they used MS media with BA at 3 mg/l and for rooting NAA at 0.3 mg/l. Budhiani (2001) demonstrated that the best combination for initiation of shoot was MS medium supplemented with 0.2 mg/l BAP + 0.002 mg/l NAA. Chaudhuri and Mukundan, (2001) reported that best multiplication of shoots was obtained on medium containing 10 mg/l BA + 160 mg/l Adenine Sulphate + 0.1 mg/l
A rapid micropropagation protocol was established for Chinese Aloe by (Liao et al., 2004) and recommended that sucrose was the most important for the bud initiation on semi-solid MS medium. Further MS medium supplemented with 2 mg/l BA + 0.3 mg/l NAA as the best medium for micro-propagation of Aloe vera. (Aggarwal and Barna 2004) provided a micro-propagation protocol for an elite selection of Aloe vera and suggested that citric acid at 10 mg/l and liquid medium improved the shoot multiplication and produced 100% rooting on hormone free agar medium. Studies conducted by (Velcheva et al., 2005) and (Debiasi et al., 2007) indicated that BA is more effective than NAA for shoot proliferation. (Sanchez et al., 1988) performed micro-propagation on vegetative meristems and found that callus induction is very difficult in Aloe barbadensis. There is only one report on callus formation and plant regeneration from seed callii of Aloe pretoriensis (Groenewald et al., 1975). (Roy and Sarkar, 1991) reported the rapid propagation by inducing shoot formation on in vitro callus cultures produced from axillary shoot segment explants taken from the underground rhizomatous stem. Polyvinylpyrrolidone (PVP) was used to reduce the secretion of phenolic substances from the explant. They used MS media supplemented with 1 mg/l 2, 4-D and 0.2 mg/l kinetin for callus induction. According to them, auxins and cytokinins are necessary for shoot proliferation.

Wang Li et al., (2001) treated the tube seedlings of Aloe vera with colchicine to form polyploid plants. The results show that the best induction rate could reach 50% after treatment with 0.06% colchicine in 12 hours.

A variety of soil mixtures are suggested for raising tissue cultured generating seedlings for hardening in greenhouse. A variety of hardening mixtures have been proposed like a mixture of soil, sand and perlite or vermiculite (Natali et al., 1990), soil and farmyard manure (Aggarwal and Barna, 2004) and coconut peat and perlite (Hashemabadi and Kaviani, 2008).